

Importance of Minor Components to the Oxidative Stability of Camelina, Chia, and Sophia Seed Oils

By

©Abrehem A. Hmed Abad

A thesis submitted to School of Graduate Studies

in partial fulfillment of requirements for the degree of

Master of Science in Food Science

Department of Biochemistry

Memorial University of Newfoundland

July 2018

St. John's Newfoundland and Labrador Canada

ABSTRACT

Camelina, chia, and sophia seed oils were evaluated for their fatty acid composition, triacylglycerol (TAG) profile, oxidative stability, and tocol contents, before and after the removal of their minor components. Samples were stripped of their minor components by using column chromatography or stripped by silicic acid-charcoal (stationary phase; 2h). All tested samples contained significant levels of α -linolenic acid and total tocol content. However, all stripped oils were devoid of any tocopherol or pigment. The oxidative state of the oils indicated that stripped oils were significantly less stable than their unstripped counterparts. Meanwhile, the photooxidative stability of oils indicated that stripped oils were more stable than their original counterparts. Involvement of chlorophylls as photosensitizers appears to be exerting a dominant effect. Therefore, these oils, similar to extra virgin olive oil, must be protected from light by using tin containers or dark bottles in order to retain their health promoting effect and prevent off-flavour development.

ACKNOWLEDGEMENTS

I take this opportunity with pride and enormous gratification to express the feelings of thanks and gratefulness from the bottom of my heart to all those who backed me up whether directly or indirectly throughout this research work.

First and foremost, I deem it a rare opportunity to pronounce my deep sense of gratitude to my thesis advisor Dr. Fereidoon Shahidi for providing me with the great opportunity to conduct my research under his supervision. His invaluable guidance, professional advice, encouragement and kind support throughout my studies is greatly acknowledged. The door to Prof. Shahidi's office was always open whenever I ran into a trouble spot or had a question about my research or writing, he steered me in the right direction whenever he thought I needed it.

I would like to extend my sincere thanks and regards to the late Dr. R. Hoover, who initially accepted me as a graduate student and provided the support needed for my research. I would also like to express my sincere appreciation to the members of my supervisory committee, Dr. Ryan Mailloux and Dr. Ross McGowan for their insights and advice. I also gratefully acknowledge the Department of Biochemistry and School of Graduate Studies at Memorial University of Newfoundland for their support as well as assistance.

I would like to thank the lab mates for their guidance and support. My special mention goes to my dear friend Dr. Priyatharini Ambigaipalan for assisting me in carrying out samples analysis and also in the analysis of results as well as constant encouragement and support. Finally, I would like to thank my family and friends for having given me the support to work on my thesis and helping me achieve my goals.

TABLE OF CONTENTS

ABSTRACT.....	ii
ACKNOWLEDGEMENTS	iv
CHAPTER 1.....	1
INTRODUCTION.....	1
CHAPTER 2.....	4
LITERATURE REVIEW.....	4
2.1. Lipid composition	4
2.1.1 Major Components.....	4
2.1.1.1 Triacylglycerols (TAG).....	4
2.1.2. Minor Components.....	6
2.1.2.1. Monoacylglycerols (MAGs), diacylglycerols (DAGs), and free fatty acids (FFAs).....	6
2.1.2.2. Phospholipids.....	10
2.1.2.3. Pigments (chlorophylls and carotenoids).....	12
2.1.2.4. Tocols (tocopherols and tocotrienols).....	16
2.1.2.5. Other phenolics.....	19
2.1.2.6. Phytosterols.....	21
2.2. Selected sources of specialty oils from oilseeds.....	23
2.2.1. Camelina Sativa (<i>Camelina sativa</i>).....	23
2.2.2. Chia (<i>Salvia hispanica</i> L.).....	27
2.2.3. Sophia <i>Descurainia sophia</i> (L.).....	29
2.3. Lipid oxidation and its mechanisms of action.....	32

2.3.1. Autoxidation.....	33
2.3.2. Photooxidation.....	39
2.4. Removal of minor components.....	44
2.5. Measurement of lipid oxidation.....	45
2.5.1. Primary products of lipid oxidation.....	46
2.5.2. Secondary products of lipid oxidation.....	47
2.6. Control of lipid oxidation.....	49
2.6.1. Synthetic antioxidants.....	50
2.6.2. Natural antioxidants.....	52
CHAPTER 3.....	54
MATERIALS AND METHODS.....	54
3.1. Materials.....	54
3.2. Methods.....	55
2.2.1. Fat Extraction.....	55
3.2.2. The removal of minor components from camelina, chia, and sophia seed oils.	55
3.2.2.1. Column chromatographic method.....	55
3.2.2.2. A novel and simplified stripping method using stationary phase material...	56
3.2.3. Analysis of camelina, chia, and sophia seed oils.....	57
3.2.3.1. Fatty acid composition.....	57
3.2.3.2. Triacylglycerol profiles.....	58
3.2.3.3. Measurement of minor components.....	59
3.2.3.3.1. Measurement of pigments (chlorophylls and carotenoids).....	59
3.2.3.3.2. Determination of tocopherols.....	59

3.2.4. Oxidative stability tests of the original seed oils and their stripped counterparts under accelerated oxidation conditions using a Schaal oven and fluorescent lighting.....	60
3.2.4.1. Determination of lipid oxidation.....	62
3.2.4.1.1. Determination of conjugated dienes (CD).....	62
3.2.4.1.2. Determination of 2-thiobarbituric acid-reactive substances (TBARS)...	63
3.2.5. Statistical analysis and data interpretation.....	64
CHAPTER 4.....	65
RESULTS AND DISCUSSION.....	65
4.1. Removal of minor components from oils using column stripping and stripping by stationary phase (2 h) methods.....	65
4.1. Characterization of camelina, chia, and sophia seed oils.....	66
4.1.1. Fatty acid composition.....	67
4.1.2. Triacylglycerol composition.....	70
4.2. Assessment of minor components in camelina, chia, and sophia seed oils.....	74
4.2.1. Assessment of tocols in the original, column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oil.....	74
4.2.2. Pigments determination in the original, column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oil.....	76
4.3. The oxidative stability of the original, column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oil.....	79

4.3.1. Autoxidation of original, column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oil and formation of primary and secondary oxidation products under Schaal oven condition.....	79
4.3.1.1. Primary products under Schaal oven condition.....	79
4.3.1.2. Secondary oxidation products under Schaal oven condition.....	82
4.3.2. Assessment of oxidative stability of original, column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oil under fluorescent light.....	84
4.3.2.1. Primary oxidation products under fluorescent light.....	84
4.3.2.2. Secondary oxidation products under fluorescent light.....	87
CHAPTER 5.....	90
5.1. The optimization of a new stripping method using stationary phase material.....	90
5.2. Assessment of minor components.....	91
5.2.1. Assessment of camelina, chia, and sophia seed oil tocols in the original, column stripped, and stripped by stationary phase (1, 2 h), and for a second time.....	91
5.2.2. Pigment determination in the original camelina, chia, and sophia seed oil, and column stripped, and stripped by stationary phase (1, 2 h), and for a second time.....	93
5.3. Assessment of minor components in corn oil, olive oil, and algal oil (DHASCO)...	95
5.3.1. Assessment of corn oil, olive oil, and algal oil (DHASCO) tocols in the original oil, column stripped, and stripped by stationary phase (1, 2 h), and for a second time	95
5.3.2. Pigment determination in the original, column stripped, and stripped by stationary	

phase (1, 2 h, and for a second time) of corn oil, olive oil, and algal oil (DHASCO).....	97
5.4. Oxidative stability of the original, column stripped, and stripped by stationary phase (2 h) of corn oil, olive oil, and algal oil (DHASCO).....	99
5.4.1. Autoxidation of the original, column stripped, and stripped by stationary phase (2 h) of corn oil, olive oil, and algal oil (DHASCO) and formation of primary and secondary oxidation products under Schaal oven condition.....	99
5.4.1.1. Primary products under Schaal oven condition.....	99
5.4.1.2. Secondary oxidation products under Schaal oven condition.....	101
5.4.2. Assessment of oxidative stability of original, column stripped, and stripped by stationary phase (2 h) of corn oil, olive oil, and algal oil (DHASCO) under fluorescent light.....	104
5.4.2.1. Primary oxidation products under fluorescent light.....	104
5.4.2.2. Secondary oxidation products under fluorescent light.....	106
CHAPTER 6.....	108
SUMMARY AND RECOMMENDATIONS.....	108
REFERENCES.....	111
APPENDIX.....	124

LIST OF TABLES

Table 2.1 Fatty acid composition (%) oil of camelina seed oil from literature sources.....	26
Table 2.2 Fatty acid composition (%) of chia seeds oil from literature sources.....	28
Table 2.3 Fatty acid composition (%) of sophia seeds from literature sources.....	31
Table 4.1 Recovery (g of resultant oil/ 100 g of oil) of stripped camelina, chia, and sophia seed oils by the column stripping and stripped by stationary phase (2 h).....	66
Table 4. 2 Composition of major of fatty acid (area percent) of non-stripped and column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oil.....	68
Table 4. 3 Triacylglycerol (TAG) composition (% of total TAG) of non-stripped <i>camelina</i> , <i>sophia</i> , and chia seed oils.....	72
Table 4. 4 Camelina, chia, and sophia seed oil tocol concentration (mg/kg of oil) in the original, column stripped, and stripped by stationary phase (2 h).....	75
Table 5. 1 Tocol concentration (mg/kg of oil) in the original, column stripped, and stripped by stationary phase (1, 2 h, and twice) of camelina, chia, and sophia seed oil.....	92
Table 5. 2 Tocol concentration (mg/kg of oil) in the original, column stripped, and stripped by stationary phase (1, 2 h, and twice) of corn oil, olive oil, and algal oil (DHASCO).....	96

LIST OF FIGURES

Figure 2.1 Chemical structure of a triacylglycerol (TAG) molecule.....	5
Figure 2.2 Chemical structures of monoacylglycerol (MAG), diacylglycerol (DAG).....	7
Figure 2.3 Chemical structures of oleic, linoleic and α -linolenic acids.....	9
Figure 2.4 Chemical structures of the main phospholipid subclasses in edible oils	11
Figure 2.5 Chemical Structures of chlorophyll	13
Figure 2.6 Chemical structures of some major carotenes.....	15
Figure 2.7 Chemical structures of tocopherols and tocotrienols	18
Figure 2.8 Chemical structures of selected polyphenols (phenolic acid: hydroxybenzoic acid; flavanoid: flavone; stilbenoid: stilbene; tannin: anthocyanidin; lignan).....	20
Figure 2.9 Chemical structures of stigmasterol, β -phytosterol, and campesterol.....	22
Figure 2. 10 Autoxidation mechanism of unsaturated lipid molecules.....	34
Figure 2.11 Autoxidation mechanism of oleate	37
Figure 2.12 Autoxidation mechanism of linoleate.....	38
Figure 2.13 Autoxidation mechanism of linolenate	36
Figure 2.14 Photooxidation mechanism of unsaturated lipid molecules.....	40

Figure 2.15 Photooxidation mechanism of oleate	41
Figure 2.16 Photooxidation mechanism of linoleate	42
Figure 2.17 Photooxidation mechanism of linolenate	43
Figure 2.18 The formation of malondialdehyde-thiobarbituric acid chromogen	48
Figure 2.19 Forming non-radical compounds.....	50
Figure 2.20 Chemical structures of common synthetic antioxidants; butylated hydroxianisole (BHA), butylated hydroxytoluene (BHT), and tert-butylhydroquinone (TBHQ).....	51
Figure 4. 1 Graphic presentation of major fatty acid composition of non-stripped, column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oils as presented in Table 4. 2.....	69
Figure 4.2 Visible spectra of pigments in seed oils (1) original oil, (2) stripped by stationary phase (2 h), and (3) column stripped.....	78
Figure 4.3 Conjugated dienes of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of camelina, chia, and sophia oil seed during a 7-day storage at 60 °C under Schaal oven conditions	81
Figure 4.4 Thiobarbituric acid reactive substances (MDA equivalents) of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of camelina, chia, and sophia oil seed during a 7-day storage at 60 °C under Schaal oven conditions	

.....	83
Figure 4.5 Conjugated dienes of camelina, chia, and sophia oil crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) during 72 h storage period under fluorescent lighting at 27 °C	86
Figure 4.6 Thiobarbituric acid reactive substances (MDA equivalents) of crude camelina, chia, and sophia oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) during a 72-hour storage under fluorescent light at 27 °C	88
Figure 5.1 Visible spectra of pigments in seed oils (1) original oil, (2) stripped by stationary phase 1 h, (3) stripped by stationary phase 2 h, (4) stripped by stationary phase (twice), and (3) column stripped.....	94
Figure 5.2 Visible spectra of pigments in seed oils (1) original oil, (2) stripped by stationary phase 1 h, (3) stripped by stationary phase 2 h, (4) stripped by stationary phase (twice), and (3) column stripped.....	98
Figure 5.3 Conjugated dienes of crude corn, olive, and algal oil (DHASCO) (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) during a 7-day storage at 60 °C under Schaal oven conditions	100
Figure 5.4 Thiobarbituric acid reactive substances (MDA equivalents) of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of corn oil, olive oil, and algal oil (DHASCO) during a 7-day storage at 60 °C under Schaal oven conditions	103

Figure 5.5 Conjugated dienes of crude corn, olive, and algal (DHASCO) (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) during 72 h storage period under fluorescent lighting at 27 °C105

Figure 5.6 Thiobarbituric acid reactive substances (MDA equivalents) of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of corn oil, olive oil, and algal oil (DHASCO) during a 72-hour storage under fluorescent light at 27 °C ...107

LIST OF ABBREVIATIONS

AOCS	American Oil Chemists' Society
APCI	Atmospheric pressure chemical ionization
BHA	Butylated hydroxyanisole
BHT	Butylated hydroxytoluene
CD	Conjugated dienes
DAG	Diacylglycerol
FAMES	Fatty acid methyl esters
FFA	Free fatty acids
GC	Gas chromatography
HPLC	High performance liquid chromatography
MDA	Malondialdehyde
MAG	Monoacylglycerol
n-3	Omega-3
n-6	Omega-6
PUFA	Polyunsaturated fatty acids
PL	Phospholipids
ROO•	Peroxyl radical
Sen	Photosensitizer
TAG	Triacylglycerol
TBA	2- thiobarbituric acid
TBARS	2-thiobarbituric acid reactive substances
v/v	Volume / volume
w/w	Weight / weight

CHAPTER 1

INTRODUCTION

Fats and oils from a wide variety of sources are important to consumers and the food industry due to their role in providing energy, essential fatty acids, fat-soluble vitamins, and other bioactive compounds. In food preparation, fats and oils are used in cooking, as a heat transfer medium, and texture modifier, among others (Salas et al., 2000). However, most of the food oils are susceptible to oxidation (deMan & deMan, 1999) due to their unsaturated fatty acids. The products and intermediates formed in oxidized oil include free radicals, primary oxidation products such as lipid hydroperoxides, and secondary oxidation products such as aldehydes, ketones, alcohols, and hydrocarbons, among other compounds, that negatively impact their flavour and aroma (Frankel, 1980).

Evans, Turton, Grant, and Dawes (1998) reported that lipid oxidation can produce toxic products that may adversely affect biological tissues. For example, linoleic acid hydroperoxides are toxic to wild-type *Saccharomyces cerevisiae* at low levels of about 0.2 mM (Evans et al., 1998). Moreover, oxidized palm oil has been shown to induce organ toxicity in kidneys, liver, and lungs of rats (Ebong, Owu & Isong, 1999). Oxidized soybean oil, lard, and particularly sardine oil increased the liver tumour development and formation of 8-hydroxy-deoxyguanosine in the liver DNA of mice (Ichinose et al., 2004). These results suggest that consuming oxidized lipids should be avoided as much as possible.

Strategies to prevent or retard lipid oxidation in food oils are a main focus of lipid research. In the past five decades, much attention has been paid to finding effective means to extend the shelf life of oils, thus the addition of antioxidants is considered as best way to delay the oxidation (Shahidi & Zhong, 2011). As lipids are more likely to oxidize during processing,

packaging, and storage, it is important to understand the mechanism of oxidation of oils in order to prevent or delay its occurrence.

Camelina, chia, and sophia seed oils may be used in different food and non-food products. In this study, the oils were extracted from their corresponding seeds. These seed oils contain oleic (18:1), linoleic (18:2) and α -linolenic (18:3) acids, and are considered a rich source of essential polyunsaturated fatty acids (PUFAs). The potential health benefits of these oils is expected to lead to their rapid commercial development and use in a variety of products for human consumption, animal feed, and cosmetic applications.

Edible oils generally consist of 95% triacylglycerols (TAGs) and 5% of non-triacylglycerols, commonly known as minor components. The minor components are primarily composed of mono- and diacylglycerols, free fatty acids, phospholipids, tocopherols, other phenolic compounds, pigments (chlorophylls, and carotenoids), and sterols (Hammilton, 1994; Shahidi & Shukla, 1996). The stability of the oils is dictated by their degree of unsaturation and positional distribution of fatty acids in the TAGs, the type and content of minor components, as well as storage conditions.

The minor components, mainly tocopherols and tocotrienols, exhibit antioxidant activity and protect the oils from oxidation. However, the minor components can either be antioxidative or pro-oxidative, depending on their chemical nature and/or storage conditions. For instance, chlorophylls are good antioxidants in the dark but act as prooxidants (photosensitizers) under the light (Wong, 2017). Therefore, these factors can contribute to the oxidative stability of edible oils (Wong, 2018).

To monitor the quality and oxidative stability of camelina, chia, and sophia seed oils, both chemical and instrumental methods may be employed. For assessing the primary oxidation products, peroxide value and/or conjugated dienes (CD) are commonly monitored. For the secondary oxidation products, thiobarbituric acid reactive substances (TBARS), individual aldehydes or other parameters may be used (Shahidi & Zhong, 2005).

The oxidative stability of camelina, chia, and sophia seed oils has not been studied. In addition, the antioxidant effects of minor components present in these seed oils have not been investigated. Furthermore, there is no literature report on the triacylglycerol profiles of these seed oils or their impact on oxidative stability. Therefore, the main purpose of this study was to assess the effects of minor components on the oxidative stability of oils under autoxidation and photooxidation conditions. The minor components were also removed using a stripping method in order to examine their effects on the oxidative stability of the oils of camelina, chia, and Sophia seeds. In addition, the fatty acid composition, TAG profile and their minor components in stripped and non-stripped oils, such as antioxidants (tocopherols) and pigments (chlorophylls and carotenoids) were also determined.

CHAPTER 2

LITERATURE REVIEW

2.1. Lipid composition

Lipids are a large group of organic compounds that occur naturally and are characterized by their solubility in nonpolar organic solvents such as hexanes, acetone, chloroform, but being insoluble in water. The hydrophobic hydrocarbon chains present in lipids may be saturated or unsaturated. Some examples of lipids include triacylglycerols, phospholipids, glycolipids, monoacylglycerols, diacylglycerols, waxes, and sterols, among others.

As mentioned in Chapter 1, edible oils primarily consist of triacylglycerols (TAGs) and minor amounts of other components, including mono- and diacylglycerols, free fatty acids, phospholipids, sterols, tocopherols, chlorophylls, and carotenoids, among others (Shahidi & Shukla, 1996). These components in each oil contribute to its oxidative stability.

2.1.1 Major Components

2.1.1.1 Triacylglycerols (TAGs)

Triacylglycerols (TAGs) or triglycerides (TGs) are molecules with a backbone of glycerol to which three acyl groups are attached. The body fat in animals and human beings, as well as those from plant origin, mainly consist of TAG. The chemical structures of TAG show that they are tri-esters of glycerol and fatty acids as depicted in Figure 2.1

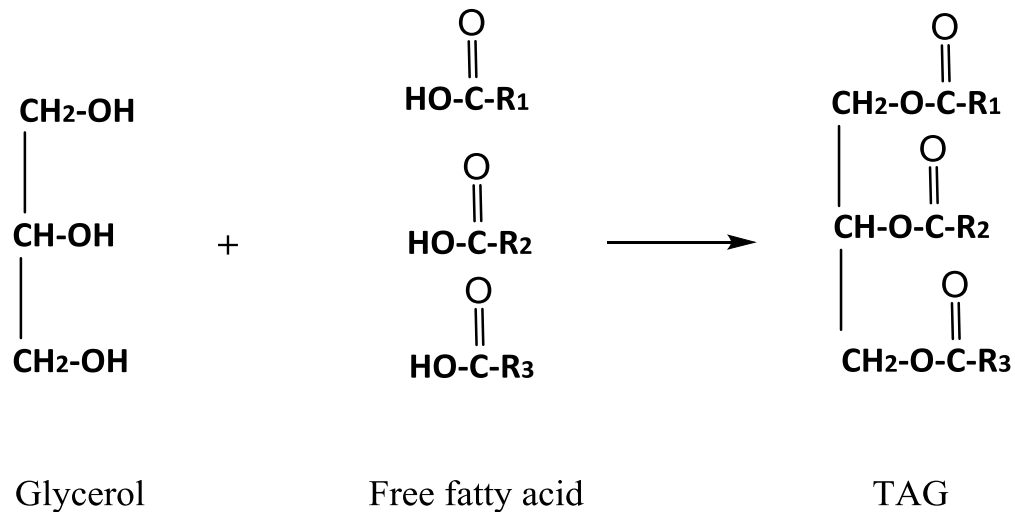


Figure 2.1 Chemical structure of a triacylglycerol (TAG) molecule

TAGs are normally stored in the adipose tissues and cells in higher eukaryotes. TAGs provide a good supply of energy attributable to the high accessibility of reduced CH groups that are helpful in energy generation processes that require oxidation. TAGs present in food are hydrolyzed immediately by salivary, gastric and pancreatic lipases and hence play an important role in metabolism (Ahmadian et al., 2007).

2.1.2. Minor Components

2.1.2.1. Monoacylglycerols (MAGs), diacylglycerols (DAGs), and free fatty acids (FFAs)

MAGs are made up of a glycerol molecule connected to a fatty acid through an ester bond (Figure 2.2). Since both secondary (*sn*-2 position) and primary alcohol groups (*sn*-1 and *sn*-3 positions) are present in glycerol, monoacylglycerols can either be 1- or 2-monoacylglycerols (Zhong, Cheong & Xu, 2014). These MAGs and DAGs are naturally present in the seed oils such as cottonseed oil, rapeseed oil, and olive oil, albeit at different levels. The most common raw materials for commercial production of MAGs are either animal fat or vegetable oil. MAGs as such, or their mixtures with DAGs, account for around 75% of the production of emulsifiers. They have wide applications in different fields such as dairy products, bakery products, confectionary, and margarine because they have conditioning, stabilizing, and emulsifying properties (Pawongrat, Xu & Aran, 2007).

Diacylglycerol (DAG) is an acylglycerol that consists of two fatty acid chains that are covalently bonded through ester linkages to a molecule of glycerol (Figure 2.2). Diacylglycerol can exist in two possible forms of 1,2-diacylglycerol and 1,3-diacylglycerol (Topham & Prescott, 2010). In the seed oils, DAGs are found as minor components (up to 10%, but usually not more than 6%). Diacylglycerols are commonly used as food emulsifiers and most often included in ice creams, beverages, candies, confections, peanut butter, bakery products, chewing gums, margarine, and whipped toppings. The relative oxidative stability of DAG and TAG oils was studied by Qi, Wang, Shin, Lee and Jang (2015). In their study, the prepared oils, soybean oil, and diacylglycerol oil were stored in the dark for 144 hours at 60°C. The oxidative stabilities of the two oils were evaluated by measuring their contents of aldehydes and peroxide values. The

results proved that the oxidation of the DAG oil was much easier than soybean oil upon storage under the conditions specified.

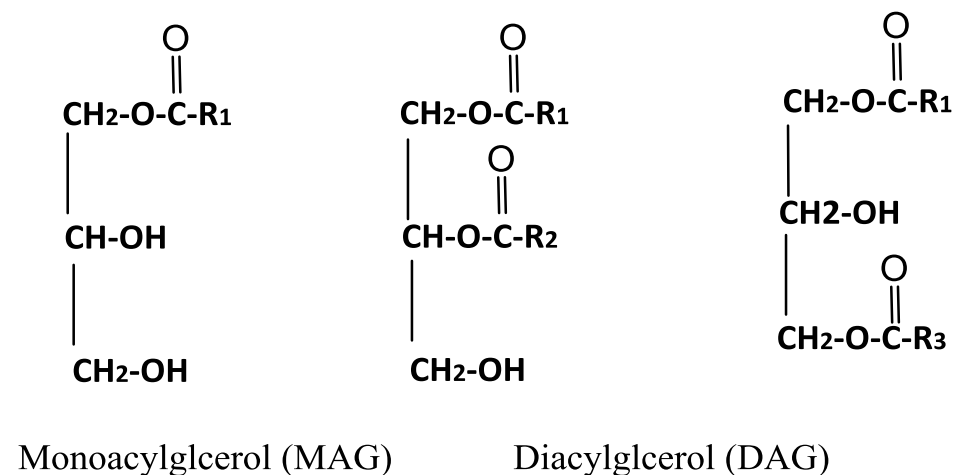
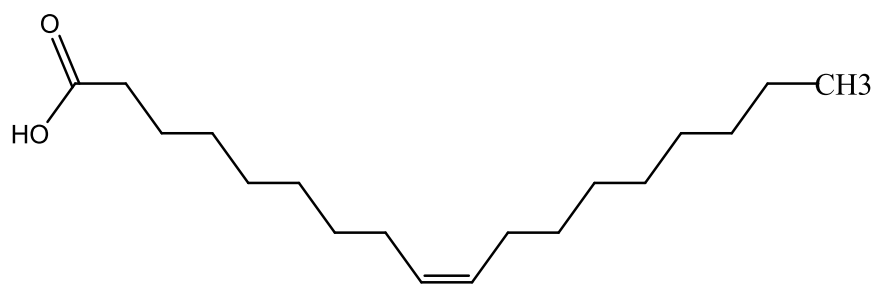


Figure 2.2 Chemical structures of monoacylglycerol (MAG), diacylglycerol (DAG) molecules.

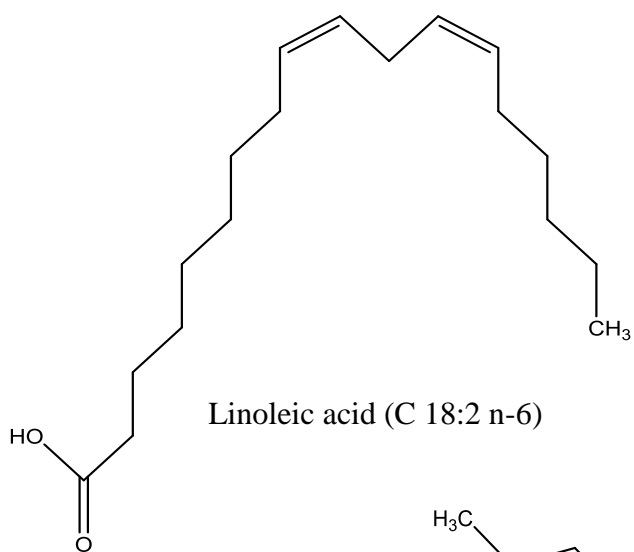
The fatty acids that occur naturally generally have an even number of carbon atoms in an unbranched chain. When fatty acids do not exist in their glycerol ester form, they are known as free fatty acids (FAAs) or non-esterified fatty acids (NEFAs) (Chaw, 2000). The processing of oil is done partly for the removal of oxidized or highly oxidizable products such as FFAs which are known to enhance or initiate oxidative reactions (Chaw, 2000).

The susceptibility of lipids towards spontaneous oxidation depends on their fatty acid profile, among other factors. Most of the seed oils are known for their high content of polyunsaturated fatty acids (PUFAs); however, they are low in saturated fatty acids. Seed oils are rich in oleic (18:1), linoleic (18:2n-6), and α -linolenic (18:3n-3) acids (Figure 2.3). The relative rate of oxidation of these fatty acids is 1:25:100 (Senanayake & Shahidi, 2002).

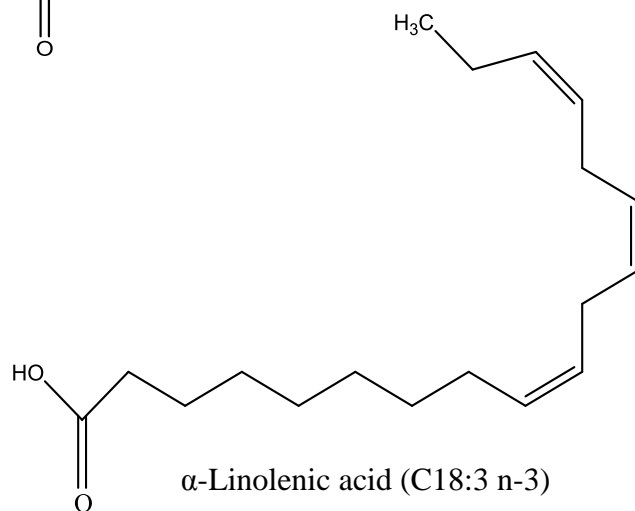
The fatty acid composition of edible oils is generally determined after their transmethylation; there are two different transmethylation methods that are commonly used: the sulphuric acid/ methanol method and the boron trifluoride (BF_3)/ methanol method (Khan & Shahidi, 2000; Budge & Parish, 2003). The FAMES are more volatile compared to the original fatty acids. The volatile FAMES are then subjected to gas chromatography (GC) to identify them by comparison with commercial standards according to their retention times. In addition to transmethylation methods, saponification value, as well as iodine value, can also be used as indicators of the fatty acids present in different oils (AOCS, 1990).



Oleic acid (C18:1)



Linoleic acid (C 18:2 n-6)



α -Linolenic acid (C18:3 n-3)

Figure 2.3 Chemical structures of oleic, linoleic and α -linolenic acids.

2.1.2.2. Phospholipids

Phospholipids (PL) are the main components of the cell membranes. The molecular structure of phospholipids consists of a phosphate group, two fatty acids, and a molecule of glycerol. The term phospholipid denotes any lipid containing a phosphate group, either mono- or diester. Some phospholipids also contain amino groups. Phospholipids are divided into two subclasses that include sphingolipids and the glycerophospholipids (Christie, 1973). Phospholipids occur in edible oils usually at levels between 0.1 and 2% but this could be as high as 10%. The main phospholipids subclasses occurring in edible oils are phosphatidic acid (PA), phospholidylethanolamine (PE), phosphatidylcholine (PC), phosphatidylinositol (PI), phosphatidylglycerol (PG), and phosphatidylserine (PS) (Figure 2.4) (Du Plessis & Pretorius, 1983; Imbs & Pham, 1995; Wanasundara & Shahidi, 1997).

Phospholipids are important factors impacting the flavour stability of edible oils because of their higher degree of unsaturation as compared to triacylglycerols (King et al., 1993). Phospholipids might act as antioxidants or prooxidants and chelating agents. The effects of total lipids, neutral lipids (NL) as well as PL extracted from fish (bluefish) have been examined using a salmon oil model system (King, Boyd & Sheldon 1992). Phospholipid fractions at 2.5 and 5% (w/w) addition levels improved the oxidative stability of this model system when incubated at 55 or 180°C. The importance of phospholipids for the vegetable oils' oxidative stability has been described by Linow and Mieth (1976), they found that the effect of tocopherol on the methyl linoleate's oxygen absorption was improved with the addition of a fraction of selected phospholipids. An antioxidant effect is provided by the phospholipids that work in synergy with tocopherols.

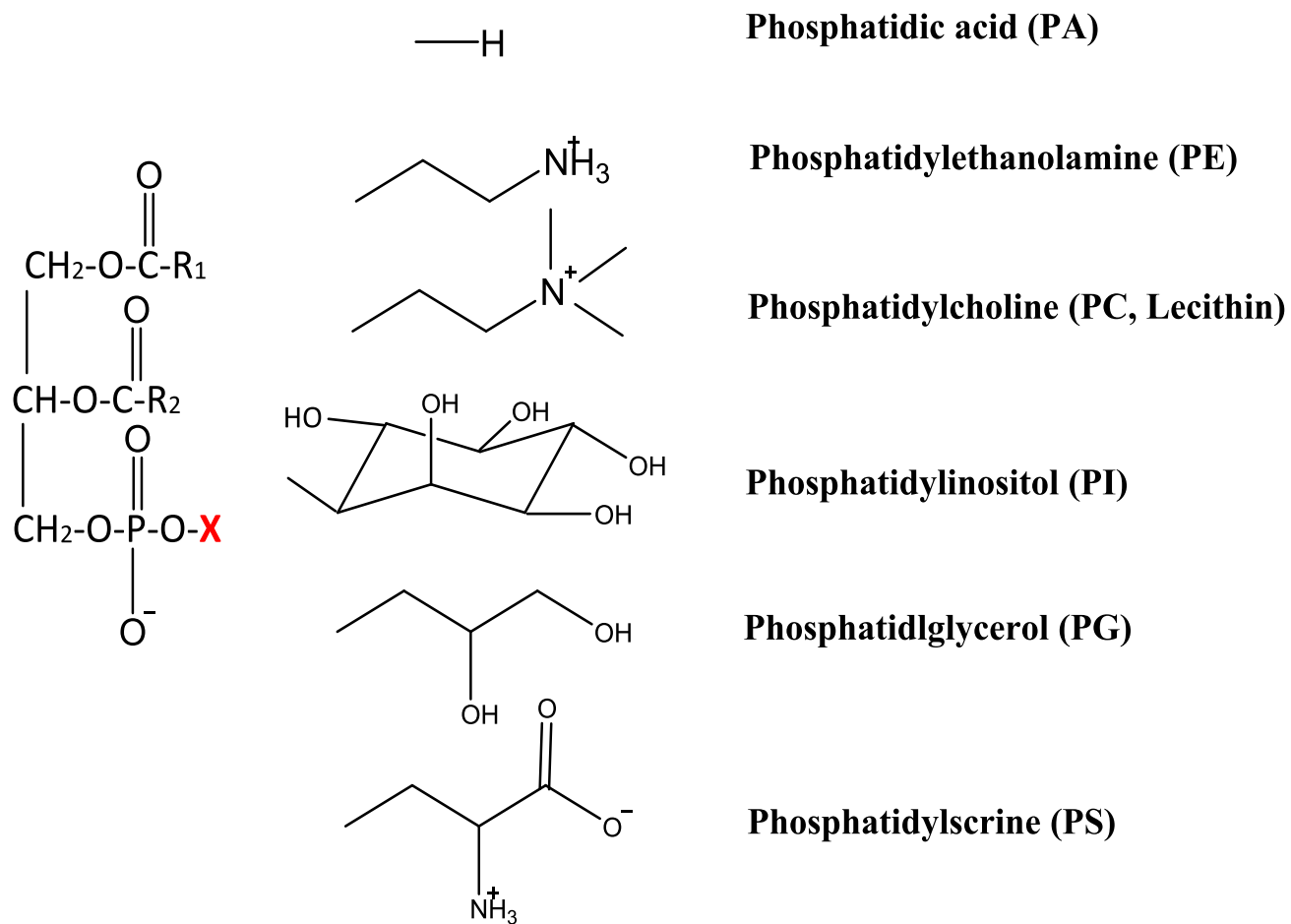


Figure 2. 4 Chemical structures of main phospholipid subclasses in edible oils (Cevc, 1993).

Hildebrand, Terao and Kito (1984) reported that soybean oil triacylglycerols' oxidative stability was improved to a certain extent by the addition of phospholipids alone. The higher the concentration of phospholipids in the oil, the higher was its oxidative stability. Addition of the phospholipids that were isolated from coconut oil using column chromatography to degummed, bleached and deodorized coconut oil in the Rancimat test at 120 °C for 5.1-32.3 h, increased the induction period of the oil. However, Nwosu, Boyd and Sheldon (1997) reported that sphingomyelin (SP), phosphatidylcholine (PC), and phosphatidylethanolamine (PE) had almost no antioxidant activity in a food grade menhaden oil, but were active in salmon oil.

Shahidi and Shukla (1996) reported that the relative antioxidant activities of commercial PL on the oxidation of salmon oil were in the order of sphingomyelin (SP)=LPC=PC=PE> PS>PI>PG> control of the salmon oil. Tocopherols together with phospholipidylethanolamine (PE) were more effective than tocopherols with phosphatidylserine (PS) or phosphatidylcholine (PC) in controlling lipid oxidation in mackerel and sardine lipids (Ohshima, Fujita, & Koizumi, 1993).

In oxidative stability of docosahexaenoic acid (DHA)-containing oils (DHA at 10.7 mol % of total fatty acids), in the form of PL, TAG, and ethyl esters (EE), Song, Inoue, and Miyazawa (1997) examined their oxidative stability in the dark at 25°C in the bulk phase. The results showed that DHA-containing oil in the form of phospholipids had more resistance to oxidative degradation than that in the forms of ethyl ester and triacylglycerol.

2.1.2.3. Pigments (chlorophylls and carotenoids)

Chlorophylls and carotenoids are the most common pigments found in edible oils (Figures 2.5 and 2.6, respectively). Chlorophylls are responsible for the green colour of most plants and plant products (Princen et al., 1995), and may also be present in some crude oils. In

crude rapeseed oil and virgin olive oil, chlorophylls are found at 5-35 and 10 ppm, respectively. During processing of the oil (specifically the bleaching process), these pigments are generally removed. Crude canola oil contains 26.2 ppm of chlorophylls but in the bleached oil, just 5% of it is left (Akoh, 2017).

In the presence of light, the chlorophylls along with their degradation products act as sensitizers. The energy is transferred to triplet oxygen present in the atmosphere which produces singlet oxygen, thus accelerating lipid oxidation. In a study conducted by Kim, Decker, and Lee (2012), the effects of chlorophyll photosensitization on oxidative stability in oil-in-water emulsions was demonstrated. They concluded that the rate of lipid oxidation was accelerated by chlorophyll photosensitization. The results also showed that the singlet oxygen's involvement in chlorophyll sensitized oil-in-water emulsions was strongly supported by the resultant volatile profiles. Carlsson, Suprunchuk and Wiles (1976) reported that it is very important to remove as much chlorophyll as possible during the refining process of the bulk oil since most retail oils are stored in transparent plastic packages.

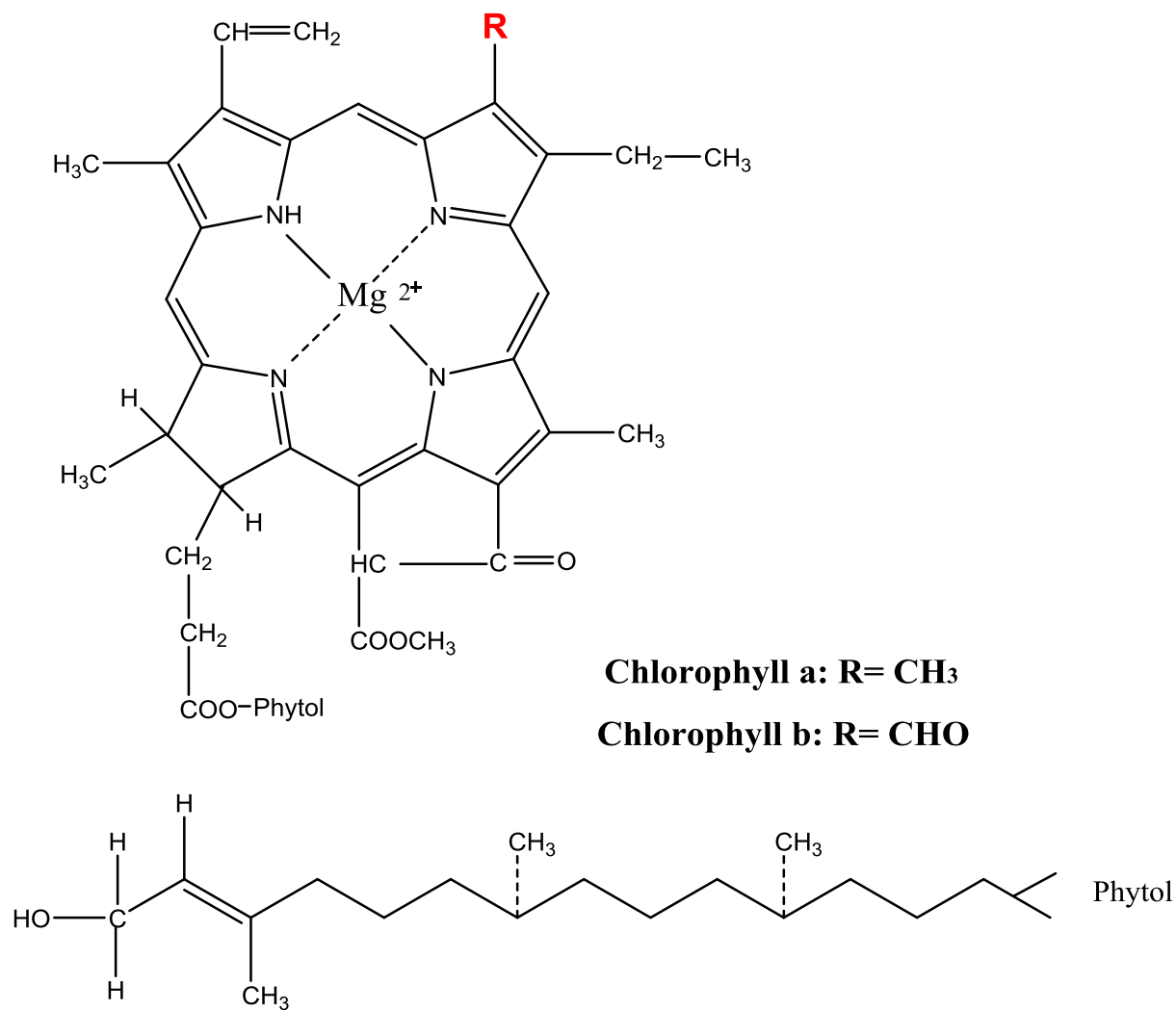
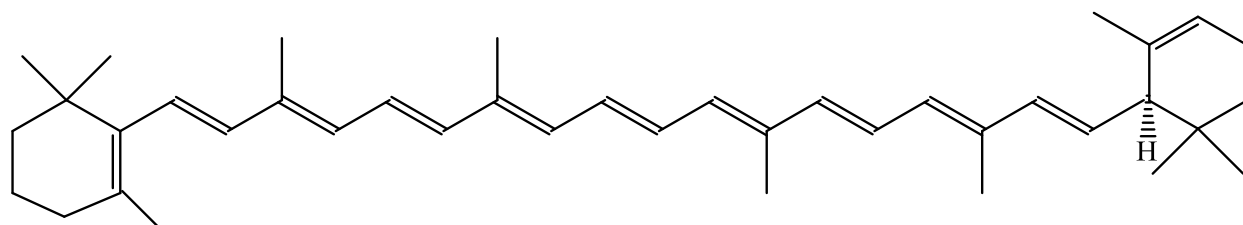


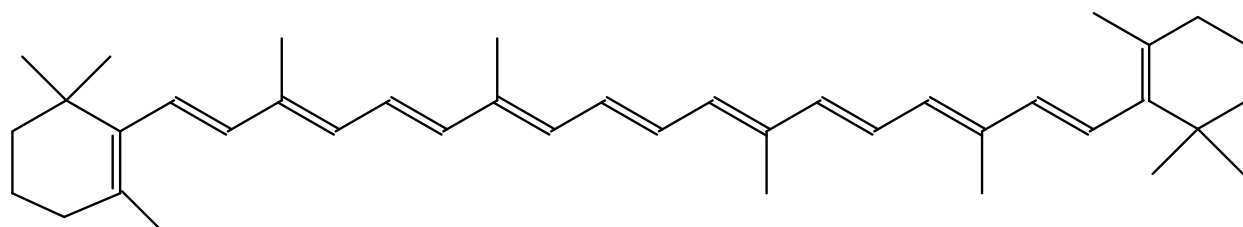
Figure 2.5 Chemical structure of chlorophylls (İnanç, 2011).

Carotenoids are tetraterpenoid compounds that are composed of isoprenoid units. The double bonds present in carotenoids are in *trans* form and are conjugated. The most common carotenoid found in oils is the beta-carotene (β -carotene; Figure 2.6). Carotenoids can degrade to colourless products at temperatures of 150°C during processing. The oxidation of lipids is slowed down by β -carotene as it filters the light, quenches singlet oxygen, and scavenges free radicals. β -carotene does not have a very high reduction potential (just 1.06V) to donate a hydrogen atom to lipid peroxy radicals or lipid radicals. However, the reduction potential of a hydroxyl radical (HO^*) is 2.31V at which β -carotene can donate a hydrogen atom and become a carotene radical. The stability of the carotene radical is better than hydroxyl radical as the unpaired electron present in carotene is delocalized through the double bond of the polyene system (Akoh, 2017). At a low concentration of oxygen, the reaction of the carotene radical takes place with lipid peroxy radicals leading to the formation of nonradical carotene peroxides.

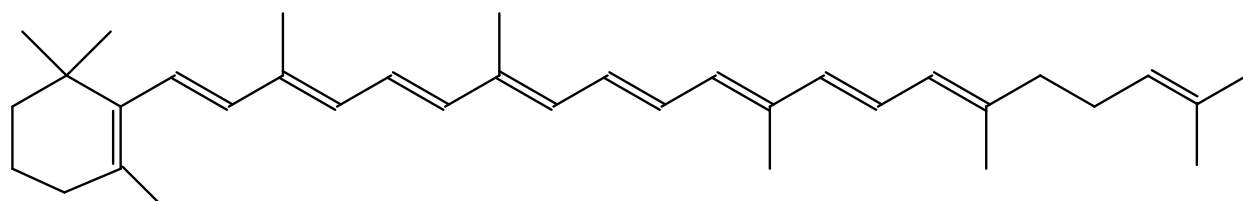
In the absence of chlorophylls, the formation of hydroperoxides is decreased by the addition of β -carotene to purified olive oil. Thus, under light, oxygen consumption in headspace in the oil at 25°C is decreased at higher concentrations of β -carotene (Akoh, 2017). In soybean oil, photooxidation was also decreased by the addition of β -carotene due to the quenching of singlet oxygen. The quenching of singlet oxygen by β -carotene occurs by energy transfer and the β -carotene's oxidation itself is not involved. The carotenoids' singlet oxygen quenching activity is increased as the number of conjugated double bonds is increased in their structures. The carotenoids' β -ionone ring's substituents also affect the quenching activity of singlet oxygen (Akoh, 2017).



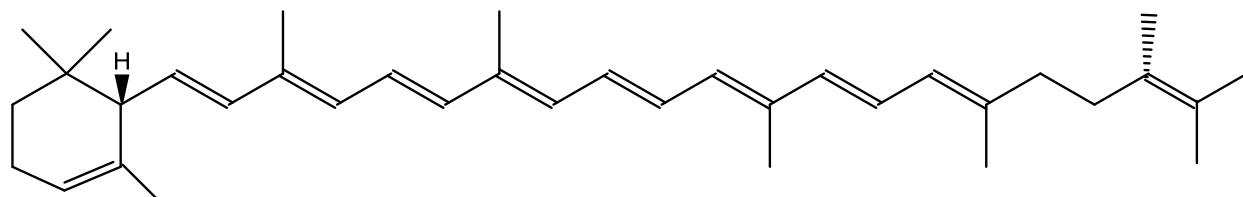
α - Carotene



β - Carotene



γ - Carotene



δ - Carotene

Figure 2.6 Chemical structures of some major carotenes.

Carotenoids' strong antioxidant activity against photooxidation and autoxidation has been well documented (Shahidi, Metusalach, & Brown, 1998; Metusalach, Brown & Shahidi, 1999). The effects of chlorophyll and β -carotene on the oxidative stability of olive oil was studied by Fakourelis, Lee and Min (1987). It was found that under the light, chlorophyll acts as a photosensitizer for the formation of singlet oxygen. The purified oil's oxidation was minimized by β -carotene due to its light-filtering effect.

2.1.2.4. Tocols (tocopherols and tocotrienols)

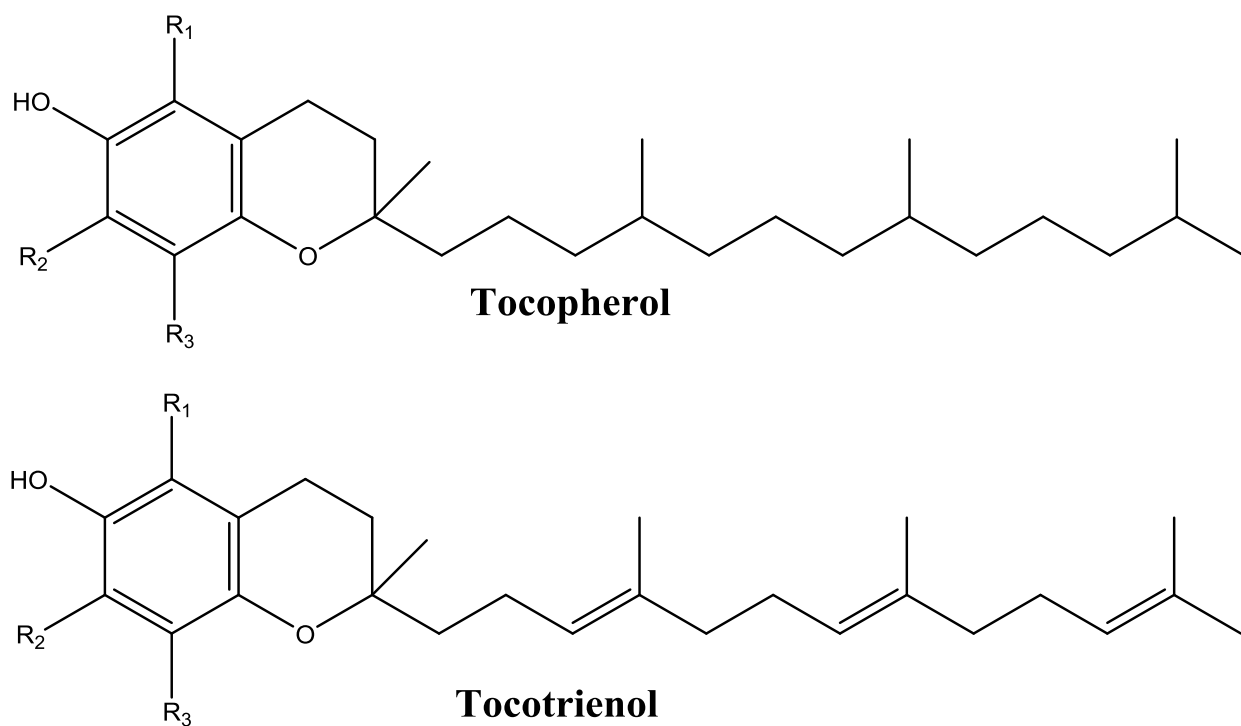
Tocopherols are a class of organic compounds found in vegetable oils as well as green leafy vegetables (Figure 2.7). There is a great utility of natural mixed tocopherols in preventing oxidation and preserving the taste of many food items that contain fats and oils. There are 4 tocopherols in edible oils, namely, α -, β -, γ -, and δ -tocopherols. Tocotrienols have three double bonds in the side chain in their structures when compared to their corresponding tocopherols. Thus, they may be considered as having unsaturated isoprenoid side chains as shown in Figure 2.7. All tocopherol and tocotrienol homologs have some antioxidant activity as they can donate a hydrogen atom to free radicals and some reactive oxygen species (ROS) via their hydroxyl group (Akoh, 2017). The α -, β -, γ -, and δ - homologs differ in their number and position of methyl groups on the chromane ring (Shahidi & Shukla, 1996; Shahidi & de Camargo, 2016).

The vegetable oils' oxidative stability is mainly determined by their fatty acids as well as the antioxidants, mainly tocopherols and other constituents that are present as unsaponifiable matter. The degree of unsaturation of oil and the position of unsaturation within a molecule of triacylglycerol determine the impact on oxidative stability. Tocopherols and tocotrienols,

especially α - and γ -tocopherols, are the main antioxidants in plant oils. Alpha-tocopherol is the major tocopherol in many edible oils as exemplified by peanut, almond, sunflower, and olive oils. However, the content of gamma-tocopherol in some edible oils such as soybean, canola, walnut, corn, and linseed oils is higher than that of alpha-tocopherol (Grilo et al., 2014; Shahidi & de Camargo, 2016). As evidenced for other phenolic compounds (de Camargo, Regitano-d'Arce, Biasoto, & Shahidi, 2014), potential health benefits of tocopherol intake are numerous and go beyond addressing any deficiency issues. Thus, in order to prevent certain types of cancer and heart disease, as well as other disorders, the actual required intake of tocols might be much higher than their recommended daily intake (de Camargo, Regitano-d'Arce & Shahidi, 2015). However in food, there is a complex phenomenon associated with the tocopherols' antioxidant behaviour because at low concentrations they act as efficient antioxidants but as their concentration increases in vegetable oils, they lose their efficacy and may even act as prooxidants at high concentrations (Kamal-Eldin, 2006).

Soybean oil is very rich in γ - and δ - tocopherols, therefore it is commonly used as a commercial source of mixed tocopherols and a source of natural antioxidants. Tocotrienols are less common compared to tocopherols. Tocotrienols can be found in palm and rice bran oils (Ko et al., 2003; Abidi, 2003). Tocols also exert a strong singlet oxygen scavenging effect and are able to prevent or control photooxidation of lipids. Jung and Min (1990) studied the effects of tocols (α -, γ - and δ -tocols) at different concentrations on the chlorophyll photosensitized oxidation of soybean oil. The results showed that α -tocopherol exhibited the highest singlet oxygen quenching activity compared to γ - and δ - tocopherols. Thus, tocols are usually used in food products deficient in natural antioxidants like butterfats, animal fats, and waxes (Byrd, 2001; Smith & Hong-Shum, 2011). As most vegetable oils naturally contain tocopherols, adding

antioxidants might lead to pro-oxidant effects. Tocopherols act synergistically with citric acid, ascorbic acid, and phospholipids in order to control lipid oxidation.



R1	R2	R3	Tocopherol	Tocotrienol
CH ₃	CH ₃	CH ₃	α-tocopherol	α-tocotrienol
CH ₃	H	CH ₃	β-tocopherol	β-tocotrienol
H	CH ₃	CH ₃	γ-tocopherol	γ-tocotrienol
H	H	CH ₃	δ-tocopherol	δ-tocotrienol

Figure 2.7 Chemical structures of tocopherols and tocotrienols (Shahidi, 1997; Shahidi & Zhong, 2010).

2.1.2.5. Other phenolics

Phenolic compounds are an important group of antioxidants that occur naturally in the plant kingdom. The antioxidant activity of the phenolic compounds arises from their unique structures that make them superior hydrogen donating compounds compared to many other molecules (Shahidi & Zhong, 2010). Phenolic compounds include phenolic acids, flavonoids, stilbenes, and lignans (Figure 2.8), among others, that act as free radical scavengers, and possibly as metal chelators, as well as reducing agents, therefore decreasing the formation of volatile decomposition products such as aldehydes and ketones that cause rancidity. Therefore, phenolic compounds exhibit great potential in inhibiting oxidation in foods as well as biological systems (Shahidi, Wanasundara & Amarowicz, 1994; Naczki & Shahidi, 2004; Shahidi & Naczki, 2004; Alamed, Chaityasit, McClements & Decker, 2009).

In general, the impact of antioxidant concentration on autooxidation rates depends on several factors such as the characteristics of the antioxidant and that of the sample being oxidized as well as the oxidation conditions (Shahidi & Naczki, 2004; Embuscado & Shahidi, 2015). Most of the time phenolic antioxidants lose their activity at high concentrations (Gorden, 1990) and behave as prooxidants by involvement in initiation and propagation reactions. Phenolic antioxidants are more effective in extending the induction period when added to oil (Mabarouk & Dugan, 1961). Therefore, antioxidants must be added to foodstuffs in early steps of processing and storage in order to achieve protection against oxidation (Shahidi & Wanasundara, 1992, Shahidi & Ambigaipalan, 2015).

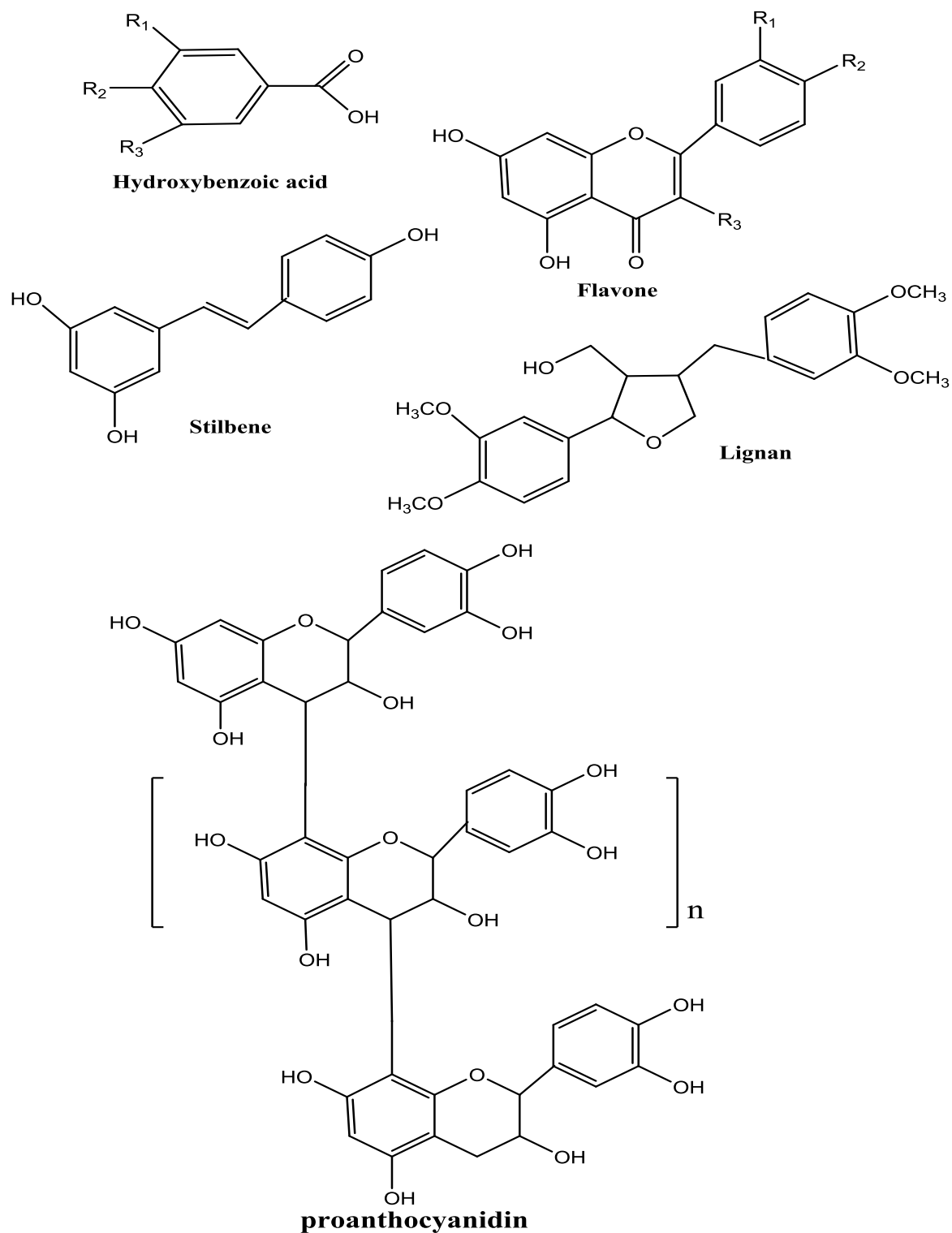


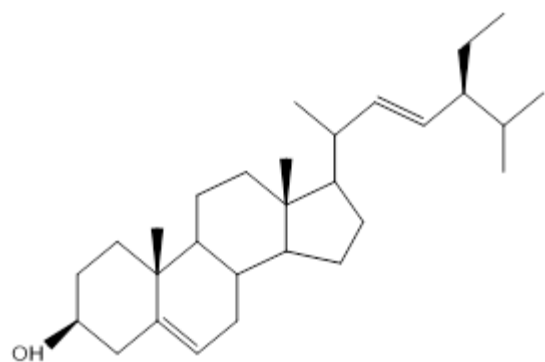
Figure 2.8 Chemical structures of selected phenolic compounds (phenolic acid, hydroxybenzoic acid; flavanoid, flavone; stilbenoid, stilbene; condensed tannin, proanthocyanidin; and a lignan).

2.1.2.6. Phytosterols

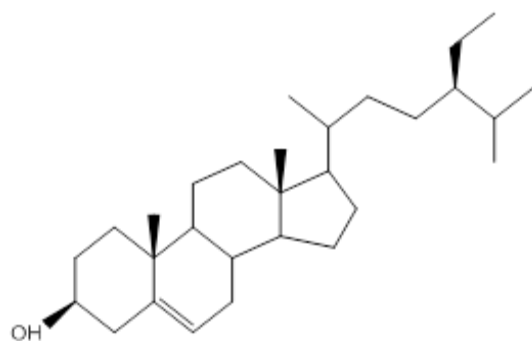
Phytosterols or plant sterols (PS) are found in cereals, seeds, and vegetable oils, among others. The molecular structures of PS are similar to that of cholesterol. Stigmasterol, β -phytosterol, and campesterol (Figure 2.9) are the most commonly found phytosterols in nature.

Phytosterols are easily oxidized and can also generate several types of hydroxy, epoxy, keto, and other derivatives which are known as phytosterol oxidation products (POPs) that may be generated when subjected to long-term storage or heat. The water content, sterol structures, composition of lipid matrix, presence of pigments, metal ions, light, and some oxidant enzymes dictate the amount of POPs that could be generated (Botelho, Galasso & Dias, 2014).

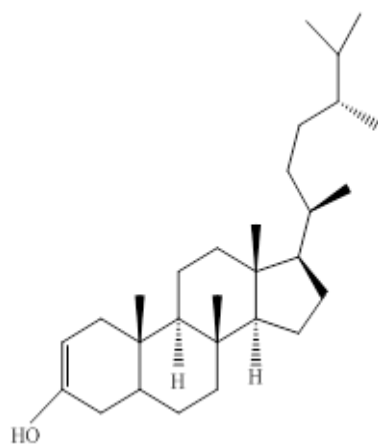
As lipid compounds, the phytosterols' oxidative stability can be defined as how well they resist the formation of POPs. The oxidative stability of phytosterols in different oils during long-term storage and processing was studied by Soupas, Huikko and Lampi (2006). The authors concluded that stored oils and fats did not represent an important source of phytosterol oxides. Thus, the formation of phytosterol oxides was not mentioned as a limiting factor in the manufacture and storage of oils and fats.



Stigmasterol



β -Phytosterol



Campesterol

Figure 2.9 Chemical structures of stigmasterol, β -phytosterol, and campesterol (Moreau, Whitaker & Hicks, 2002).

2.2. Selected sources of specialty oils from oilseeds used in this study

2.2.1. Camelina Sativa (*Camelina sativa*)

Camelina sativa (*Camelina sativa*) is an ancient oilseed crop that is also known by different common names such as linseed dodder, gold of pleasure, or Dutch flax. It is a cruciferous oilseed plant, belonging to the cruciferae (*Brassicaceae*) family which includes canola, rapeseed, mustard, crambe and also vegetables like broccoli, cabbage, and cauliflower (Plessers et al., 1962; Shahidi, 1990; Putnam, Budin, Field & Breene, 1993; Zubr, 1997; Blackshaw et al., 2011; Li & Mupondwa, 2014). Camelina crop is native to Central Asia and Northern Europe and has been cultivated as an oilseed crop. It was a vital oil crop amid the Bronze and Iron Ages and it is still not clear why it was gradually supplanted in the Middle Ages and onwards (Abramovic & Abram, 2005). It was believed that seeds of camelina were consumed along with seeds such as flaxseeds and cereals primarily in the form of bread and porridge (Zubr, 1997).

Camelina has a remarkably short growing season of 85-100 days (Moser, 2010). The crop has a few positive agronomic characteristics as it is tolerant to insects and weeds, exceptionally versatile to the atmosphere and soil type, does not require high contributions of pesticides and develops well in semiarid areas (Abramovic, Butinar & Nikolic, 2007; Zubr, 1997).

The yield of camelina seeds in various states of the USA varies and is 700-1600 kg/ ha in Montana (McVay & Lamb, 2008), 600-1700 kg/ ha in Minnesota (Putnam et al., 1993), 720-2000 kg/ ha in North Dakota, and about 1000 kg/ ha in Arizona (French, Hunsaker, Thorp & Clarke, 2009). Additionally, the yield of camelina is 2600-3300 kg/ ha in Denmark (Zubr, 1997) and 1500-3250 kg/ ha in Austria (Vollmann, Damboeck, Eckl, Schrems & Ruckenbauer, 1996;

Vollmann, Moritz, Kargl, Baumgartner & Wagentristl, 2007). Globally the yield averages from 336 to 2240 kg /ha with an oil content of 35-45%. Thus, a yield of 106 to 907 litres of oil for each hectare (Moser, 2010)

The qualities of camelina contribute to an increasing interest in its use as an oilseed for both non-food and food purposes, because of its relatively high oil content and its desirable fatty acid composition (Putnam et al., 1993; Budin et al., 1995; Abramovic & Abram, 2005; Abramovic et al, 2007; McVay & Lamb, 2008). The oil is golden yellow in colour with a mild nutty and a characteristic mustard-like aroma. Abramovic and Abram (2005) reported the physical properties of camelina oil such as its iodine value of 105 (g I₂/100 g oil), its refractive index of 1.4756 (at 25° C), its saponification value of 187.8 (mg KOH/g oil), and its density of 0.92 g/mL (at 25 ° C).

Camelina is considered a new oilseed in North America that may have a promising future in western Canada. Oil of camelina is an important edible oil in the global market due to its desirable nutritive value arising from its abundance of essential fatty acids with approximately 45% polyunsaturated fatty acids (PUFAs), 35% monounsaturated fatty acids (MUFAs), 10% saturated fatty acids (SFAs), and less than 10% free fatty acids (FFAs). It also contains tocopherols, terpenes, sterols and volatiles (Das, Berhow, Angelino & Jeffery, 2014; Rahman, de Camargo & Shahidi, 2018). The most important essential fatty acids such as linoleic acid (18:2), and α -linolenic acid (18:3 n-3), as well as monoenes such as oleic acid (18:1 n-9), and gondoic acid (20:1 n-9) are found in the oil (Budin et al., 1995; Zubr & Matthaus, 2002; Eidhin, Burke, Lynch & O'Beirne, 2003; Abramovic and Abram, 2005; Frohlich & Rice, 2005; Zubr, 2009). Table 2.1 shows the fatty acid composition of camelina oil originating from various countries.

From a nutritional point of view, camelina oil, which is rich in essential fatty acids, may have a significant effect on the reduction of cholesterol as well as TAGs. Eidhin, Burke, Lynch, and O'Beirne (2003) concluded that the camelina oil diet increased long-chain ω -3 PUFAs, in particular, eicosapentaenoic acid (EPA), in addition to improving the ratio of ω -6/ ω -3 fatty acids in plasma. The total content of tocopherols in camelina oil was reported as 789-821 mg/100 g (Eidhin, Burke & O'Beirne, 2003). Currently, camelina seed oil may be used as a natural source of omega-3 fatty acids and antioxidants (mainly tocopherols) in food processing (Zubr, 1997).

Table 2.1 Fatty acid composition (%) oil of camelina seed oil from the literature

Content of Fatty acid %

Fatty acid	Leonard (1998)	Zuhr & Matthaus (2002)	Eidhin et al. (2003)	Abramovic & Abram (2005)	Moser & Vaughn (2010)	Hrastar, Cheong, Xu, Miller & Kosir (2011)
Palmitic (16:0)	5.3	5.4	5.5	6.4	6.8	5.35
Stearic (18:0)	2.5	2.5	2.3	2.6	2.7	2.33
Oleic (18:1)	12.6	14.9	14.9	17.4	18.6	14.33
Linoleic (18:2n-6)	15.6	15.2	15.8	16.9	19.6	16.28
α -Linolenic (18:3n-3)	37.5	36.8	38.9	35.2	32.6	35.53
Arachidic (20:0)	1.2	1.3	0.4	1.24	1.5	1.33
11-Eicosenoic (20:1) [gondoic]	15.5	15.5	16.2	14.9	12.4	14.60
Eicosadienoic (20:2)	2.0	1.9	2.1	2.1	1.3	1.87
Erucic (22:1)	2.9	2.8	2.4	1.6	2.3	2.55

2.2.2. Chia (*Salvia hispanica* L)

There are around 900 species in the family Lamiaceae, under the *Salvia* genus, and these are utilized around the world as folk medicines and flavouring agents (Lu & Foo, 2002). The plant is an annual herb flowering in summer, reaching about one metre in height with serrated leaves of 4-8 cm long and 3-5 cm wide with hermaphrodite flowers (USDA, 2011). The seeds of the species *Salvia hispanica* L., generally known as "chia sage", "chia", and "Spanish sage", were an essential staple food, medicine, and source of oil in pre-Columbian ages (Reyes-Caudillo, Tecante & Valdivia-López, 2008). Southern Mexico and northern Guatemala are the native places for the chia plant (Ayerza, 1995). It is marketed in South America and Canada as a health food commodity (Ayerza & Coates, 2011; Segura-Campos et al., 2014). Chia was approved as a novel food by the European Union (2009) and can also be used up to 5% in bread-making (Commission of the European Communities, 2009; Rahman, de Camargo & Shahidi, 2017). Furthermore, some countries use the seeds as a refreshing material after soaking them in fruit juice or water (Cahill, 2003). Chia seeds are composed of lipid (30-33%), protein (15-25%), carbohydrate (26-41%), dietary fibre (18-30%), and ash (4-5%) (Ixtaina, Nolasco & Tomas, 2008; Porras-Loaiza, Jiménez-Munguía, Sosa-Morales, Palou & LópezMalo, 2014). It also contains a high amount of α -linolenic acid (C18:3n-3, ALA) and vitamins (Rincón-Cervera et al., 2016). Table 2.2 shows some studies in various countries on the fatty acid composition of chia oil. Valenzuela et al. (2015) reported that oil of chia seeds intake leads to conversion of its α -linolenic acid to eicosapentaenoic acid of erythrocytes in pregnant women and increase of the docosahexaenoic acid in their milk.

Table 2.2 Fatty acid composition (%) oil of chia seeds from literature sources

Fatty acid	Content of Fatty acid %				
	Ayerza (1995)	Ayerza & Coates (2004)	Chicco, D'Alessandro, Hein, Oliva & Lombardo (2009)	Ixtaina, Nolasco & Tomás (2012)	da Silva Marineli, Moraes, Lenquiste, Godoy, Eberlin & Maróstica Jr (2014)
Palmitic (16:0)	6.2	7.25	6.60	7.2	7.07
Stearic (18:0)	3.3	3.40	2.80	3.8	3.36
Oleic (18:1)	7.3	3.40	6.80	5.2	7.04
Linoleic (18:2n-6)	19.8	18.0	18.60	19.1	18.23
α -Linolenic (18:3n-3)	63.4	63.2	64.60	64.7	62.80

In addition, chia seeds and their oil contain a high amount of natural antioxidants such as tocopherols, and carotenoids, as well as phytosterols (Ixtaina et al., 2011). The phenolic compounds in chia include caffeic acid, chlorogenic acid, myricetin, kaempferol, and quercetin (Reyes-Caudillo et al., 2008; Capitani Spotorno, Nolasco & Tomás, 2012), which play an important role in protecting against many diseases and promoting health (Nijveldt et al., 2001).

2.2.3. Sophia *Descurainia sophia* (L.)

Descurainia Sophia L., commonly known as flixweed, is a member of the family Brassicaceae and is also a widely occurring annual weed (Li, Liu, Dong, Xu, Zheng & Shan, 2010; Rahman, de Camargo & Shahidi, 2018). It is native to South America, South Africa, Asia, southern Europe, and New Zealand (Aghaabasi & Baghizadeh 2012). It is widely distributed in Iran especially in the northeast regions. Sophia seed is very small and brown or dark yellow bearing an uneven surface in a stretched oval form (Ara, Jowkarderis & Raofie, 2015). In Canada, it is found throughout the Canadian prairies and it is one of the most abundant weeds (HadiNezhad, Rowland & Hosseinian, 2015).

Sophia has traditionally been used as a folk medicine in China, Iran, and India (Khan et al., 2012). According to traditional Iranian medicine knowledge, sophia has long been used for some medicinal purposes such as promoting urination, an antipyretic, an appetizer and a treatment for measles (Daryaei, 2007). Sophia plants produce a very large number of seeds with high oil yield. Sophia produces about 2,600-3,000 kg seeds/ ha, which is nearly four times that of canola (Li, Liu, Khan, Kamiya & Yamaguchi, 2005, Ahmadi & Shahmir, 2016). The seeds of sophia contain 33% oil, 28% protein, and 4% ash (WHO, 1997). Furthermore, the seed is a good

source of bioactive compounds like phenols, flavonoids and phenolic acids (HadiNezhad et al., 2015).

Sophia seed has been considered as potential source of oil for industrial utilization (Peng, Yi, Fu-Li & Ze-qü (1997). However, the application of the sophia seed oil in the food industry has not been studied in any detail. The oil of sophia is a good source of PUFAs such as α -linolenic (ω 3) and linoleic (ω 6) acids (HadiNezhad et al., 2015), and thus can be considered a functional or healthy oil. Table 2.3 presents a summary of the fatty acid composition of sophia seed oil from different studies. Sophia seed oil has a similar fatty acid profile to canola oil with α -linolenic acid (18:3n-3) as its most prevalent fatty acid (Peng et al., 1997). Genetic as well as environmental factors can significantly affect the fatty acid composition of different sophia varieties (HadiNezhad et al., 2015).

Table 2.3 Fatty acid composition (%) oil of sophia seeds from literature sources

Fatty acid	Fatty acid proportions, weight %				
	Dolya, Shkurupii, Podzolkova & Kaminskii (1973)	Bekker, Ul'chenko & Glushenkova (2005)	Guan, Jiang, Xin & Zhang (2007)	Maršalkienė, Sliesaravičius, Karpavičienė, & Dastikaitė (2009)	Ahmadi & Shahmir (2016)
Palmitic (16:0)	7.4	6.2	5.79	5.7	6.2
Oleic (18:1)	11.2	10.2	25.5	11.0	10.82
Linoleic (18:2n-6)	16.3	15.9	17.76	18.3	16.4
α -Linolenic (18:3n-3)	46.02	37.1	25.35	42.1	39.4
Arachidic (20:0)	8.2	12.1	-	1.2	1.66
11-Eicosenoic [gondoic] (20:1)	0.89	2.1	10.51	8.2	8.84
Eicosadienoic (20:2)	0.72	2.3	-	-	1.18
Erucic 22:1	6.45	10.9	8.2	7.5	8.84

2.3. Lipid oxidation and its mechanisms of action

Oxidation of lipid is responsible for quality deterioration of food as well as the integrity of the biological systems (Shahidi & Zhong, 2010). Several changes may occur in food due to oxidation that lead to the development of off-flavours, loss of nutrients, and might result in the formation of toxic compounds, therefore making the lipid or food which contains lipid unsuitable for consumption (Beckman & Ames, 1998). Oxidation *in vivo* has destructive cellular effects associated with many diseases such as atherosclerosis, cancer, aging, and inflammation, among others (Floyd & Hensley, 2002). Thus, lipid oxidation is a major concern for food scientists and nutritionists (Aruoma, 1998).

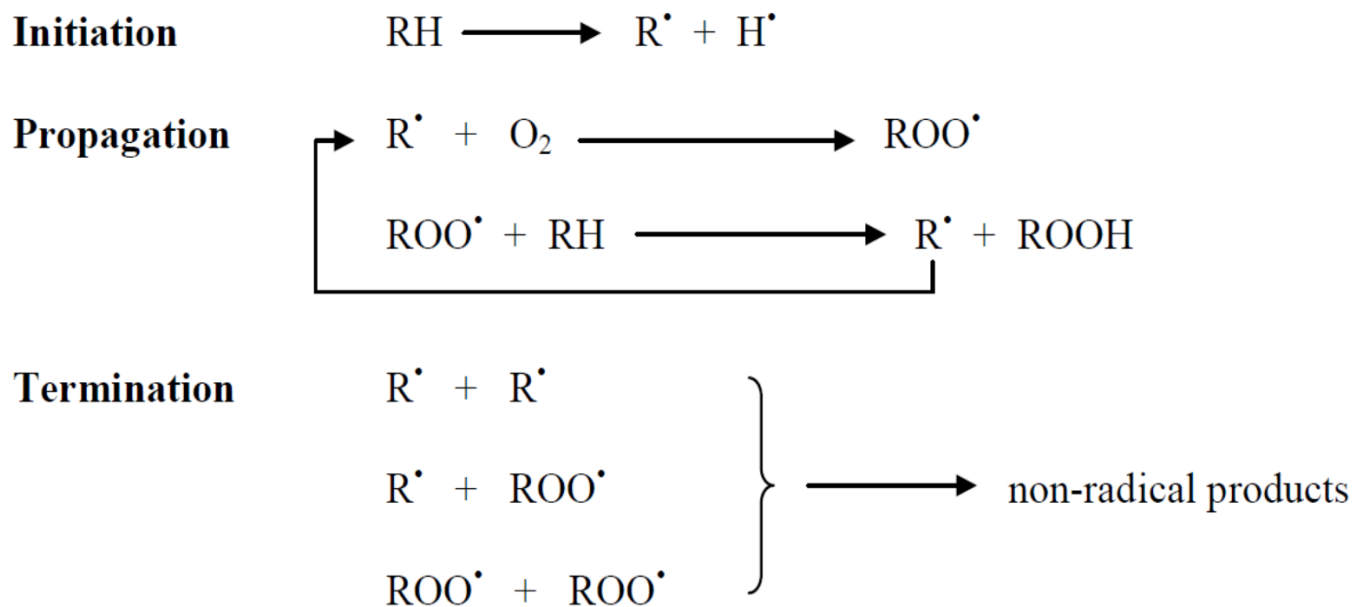
The use of antioxidants, whether natural or synthetic, for preventing and controlling lipid oxidation is practiced in order to minimize oxidative deterioration of foods. Thus, the antioxidant strategy has successfully been used in the food industry for quality preservation of products (Shahidi & Zhong, 2010). In fact, lipids are susceptible to oxidation especially in the presence of catalytic systems like heat, light, metal ions and enzymes, thus leading to complex processes of photooxidation, autoxidation, enzymatic or thermal oxidation (Shahidi & Zhong, 2010).

Many edible oils contain unsaturated fatty acids such as oleic, linoleic, and linolenic acids. The susceptibility to oxidation depends on the degree of unsaturation, therefore linolenic acid with 3 double bonds oxidizes about 25-100 times faster than oleic acid that has one double bond (Labuza & Dugan Jr, 1971). Although several types of oxidation may occur in foods, in this thesis, only those of autoxidation and photooxidation were studied and reported.

2.3.1. Autoxidation

Autoxidation is a spontaneous free radical chain reaction between molecular oxygen and unsaturated fatty acids which occurs through three steps of initiation, propagation, and termination (Frankel, 1984). In the first step, the reaction starts between atmospheric oxygen and unsaturated lipids (RH) in the presence of an initiator, such as light, heat, metal catalyst and/or UV radiation. The activation energy necessary is about 35 kcal/mol, for the formation of free radicals for this first step (initiation). Unsaturated lipid molecules (RH) lose a hydrogen atom from the allylic or bis-allylic methylenic carbon of an unsaturated fatty acid to produce a lipid free radical ($R\bullet$) that reacts with oxygen to afford a peroxy radical ($ROO\bullet$). Thereafter, a propagation step starts, where $ROO\bullet$ abstracts a hydrogen atom from another lipid molecule to form a hydroperoxide ($ROOH$) and also another lipid free radical, $R\bullet$. This reaction can end by the combination of two radical species to form a non-radical product, known as the termination step (Figure 2.10).

The primary products of autoxidation are known as lipid hydroperoxides ($ROOH$); these components have no colour or odour, and thus they have no adverse effect on the flavour quality of food. However, hydroperoxides are unstable and tend to break down and form secondary oxidation products such as aldehydes, ketones, hydrocarbons, alcohols, esters, and volatile organic acids. These secondary products have undesirable flavours and negatively affect the quality of lipids and lipid-containing foods (Figure 2.10).



RH refers to any unsaturated fatty acid and **H** is a hydrogen atom.

Figure 2. 10 Autoxidation mechanism of unsaturated lipid molecules (Shahidi & Zhong, 2005).

To illustrate these steps, for example, autoxidation of linolenate, a hydrogen atom is abstracted from bis-allylic methylene on carbon number 11 and carbon number 14. (Figure 2.13). After that, two radicals are formed, which in turn react with O₂ at the end positions to produce a mixture of 9-, 12-, 13-, and 16-conjugated diene-triene hydroperoxide isomers (Frankel, 1984). Frankel et al. (1977), as well as Chan and Levett (1977), reported that by using both HPLC and GC-MS, external 9- and 16-hydroperoxides were formed more rapidly than internal 12-, 13-hydroperoxides, possibly due to their tendency to cyclize.

The autoxidation of oleate proceeds similarly; oleate has a hydrogen atom abstracted from carbon 8 and carbon 11 to produce a mixture of 8-, 9-, 10- and 11-hydroperoxides (shown in Figure 2.11) in different proportions; linoleate's hydrogen is abstracted from carbon 11 only, as shown in Figure 2.12 and forms an equal amount of conjugated 9- and 13-hydroperoxide isomers (Frankel, 1984). As mentioned earlier, these hydroperoxide isomers are unstable and could decompose to secondary oxidation products which have off-flavour notes. The decomposition of hydroperoxide starts by a homolytic cleavage of the weak oxygen-oxygen bonds, yielding a hydroxyl and an alkoxyl radical. The alkoxyl radical subsequently decomposes by carbon-carbon cleavage to produce aldehydes, ketones, alcohols, and hydrocarbons, among others (Frankel, 1984).

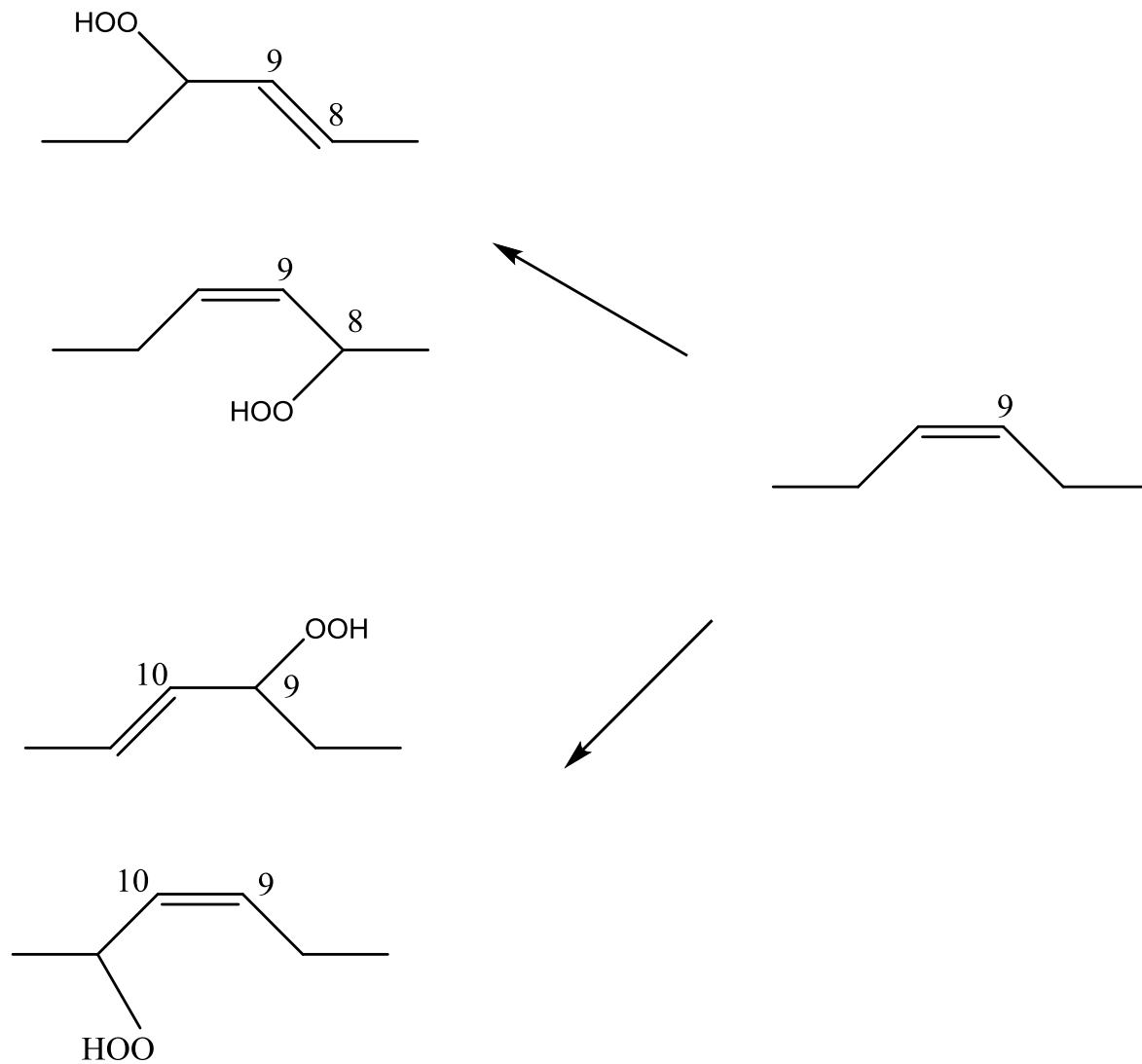


Figure 2.11 Autoxidation mechanism of oleate (Frankel, 1984).

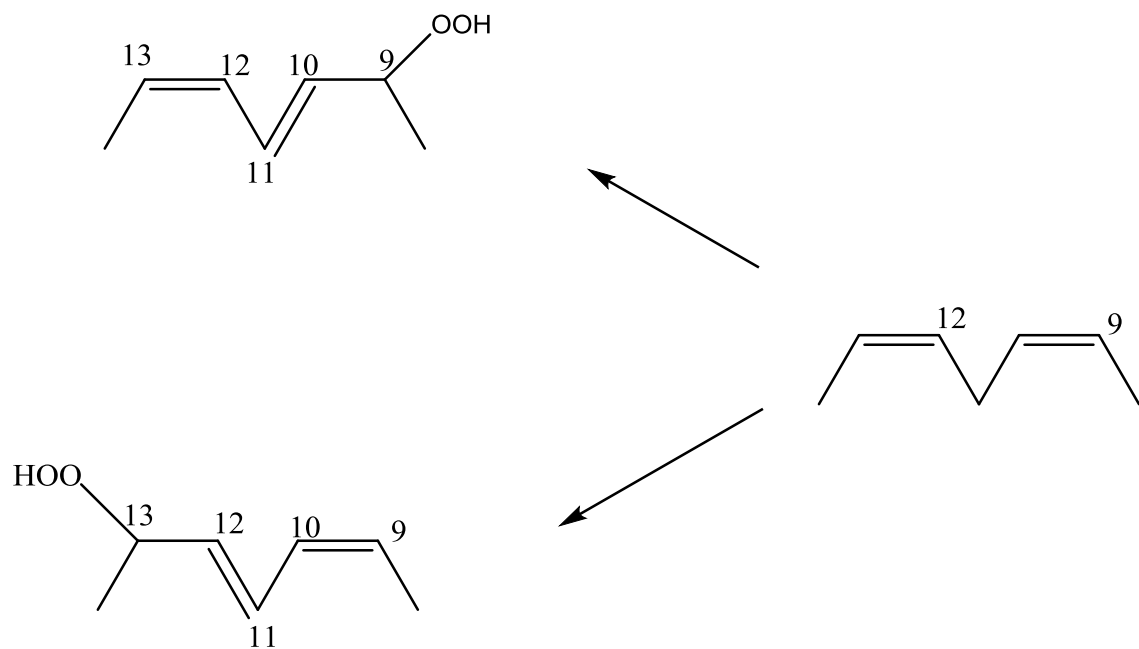


Figure 2.12 Autoxidation mechanism of linoleate (Frankel, 1984).

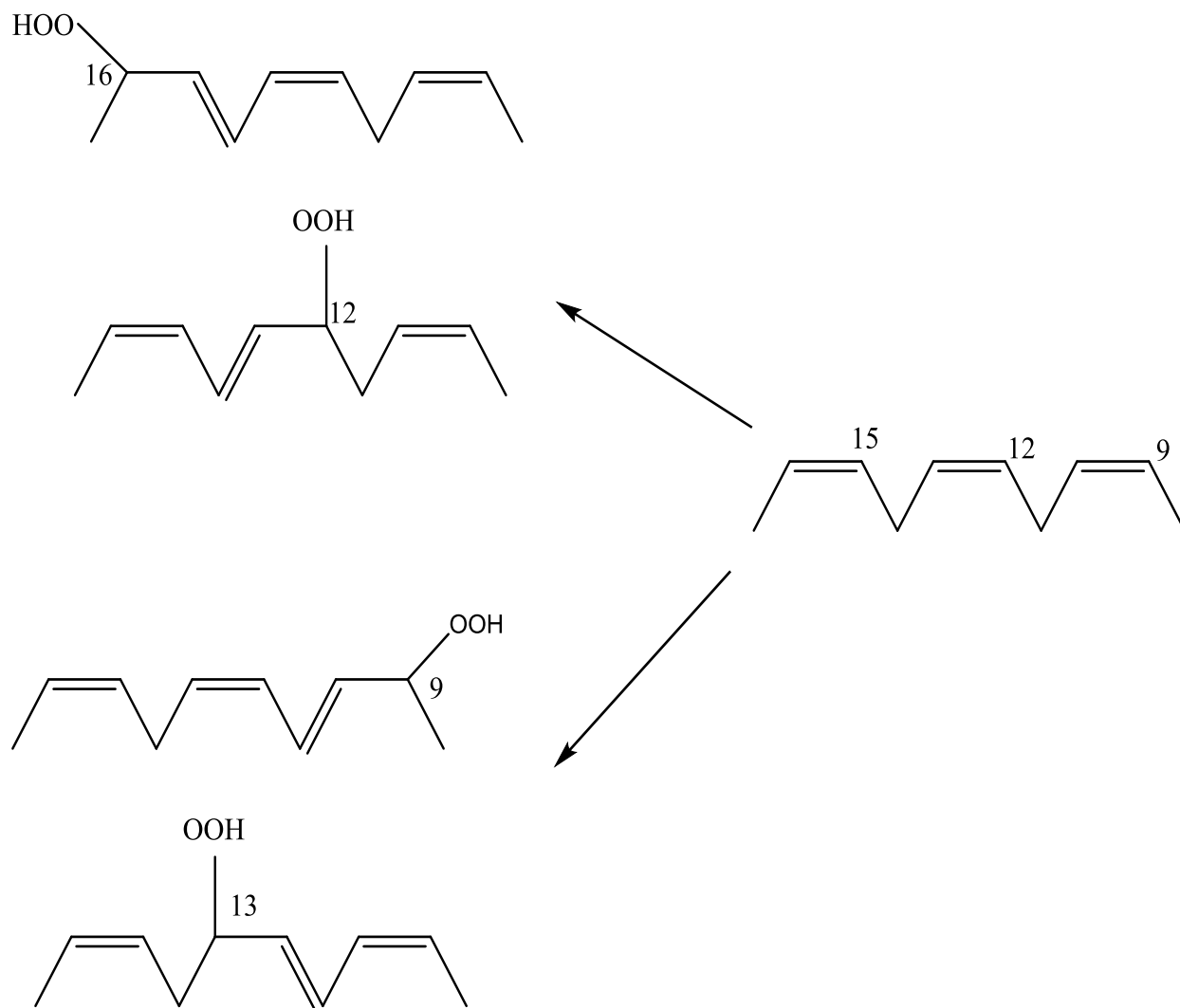


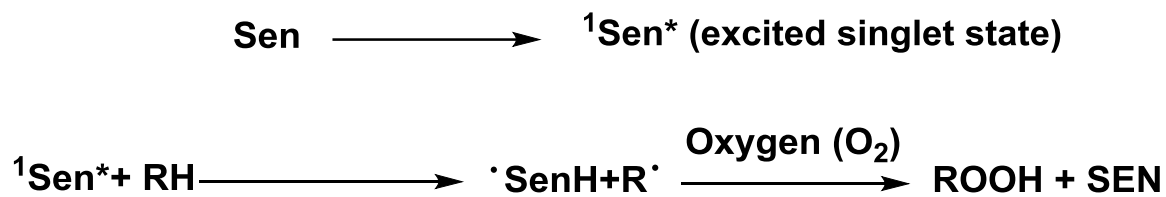
Figure 2.13 Autoxidation mechanism of linolenate (Frankel, 1984).

2.3.2. Photooxidation

Photooxidation occurs in the presence of a photosensitizer (sen) and visible light. The natural pigments in edible oils include chlorophyll, pheophytin, flavin, myoglobin and other haem-containing compounds that might absorb visible light energy efficiently because of their conjugated (double bonds), and form an excited singlet sensitizer. In addition, oxygen (triplet and singlet) is involved in this process (King, Hahm & Min, 1993). Singlet oxygen ($^1\text{O}_2$) can directly react with unsaturated fatty acids by non-radical pathways. The singlet oxygen is the primary reactive oxygen species (ROS), which reacts about 1500 times faster than triplet oxygen in terms of the oxidation reaction, as in the case of linoleate (Min & Boff, 2002).

There are two pathways proposed for photosensitized lipid oxidation. In the first pathway type 1, exposure to a light-excited singlet photosensitizer could directly interact with lipid molecules and then return to the ground state. In the second pathway, type 2, an excited photosensitizer could react with triplet oxygen to form singlet oxygen, which leads to oxidation (Figure 2.14). From the Scheme shown below, it is obvious that type 1 photooxidation forms lipid hydroperoxide via a free radical. For type 2 photooxidation, highly electrophilic singlet oxygen could attack directly the double bonds on unsaturated fatty acids, thereby producing lipid hydroperoxides.

Type (1)



Type (2)

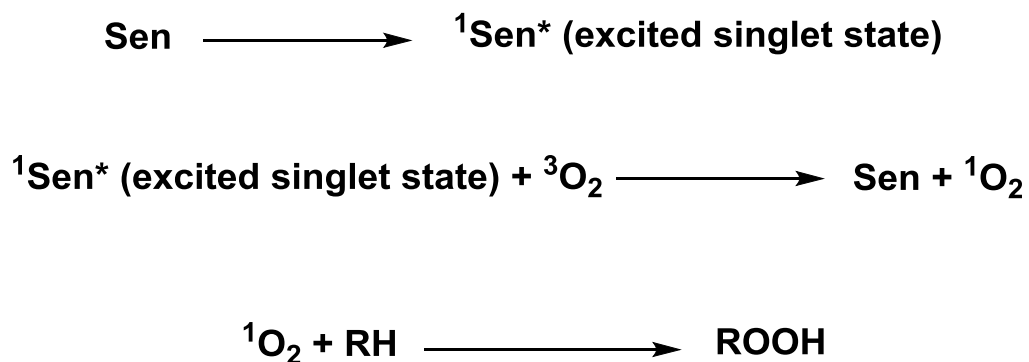


Figure 2.14 Photooxidation mechanism of unsaturated lipid molecules (Shahidi & Zhong, 2010).

Hydroperoxides are formed during singlet oxygen attack on each unsaturated carbon with a shift of the double bonds in the molecules, and thus, oleate forms 9- and 10- hydroperoxides (shown in Figure 2.15); linoleate forms 9- and 13- conjugated diene hydroperoxides as well as 10- and 12- unconjugated diene hydroperoxides (Figure 2.16); while linolenate produces 9-, 12-, 13- and 16-isomers as well as 10- and 15-isomer (Figure 2.17) that are not all the same as the products of autoxidation.

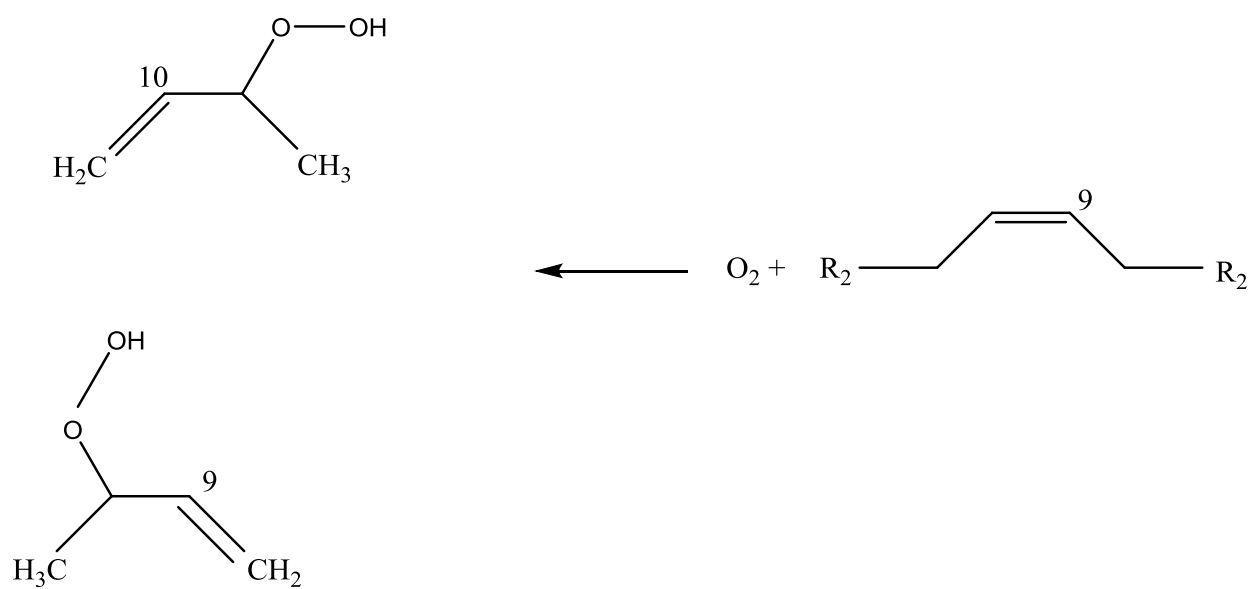


Figure 2.15 Photooxidation mechanism of oleate (Frankel, 1984).

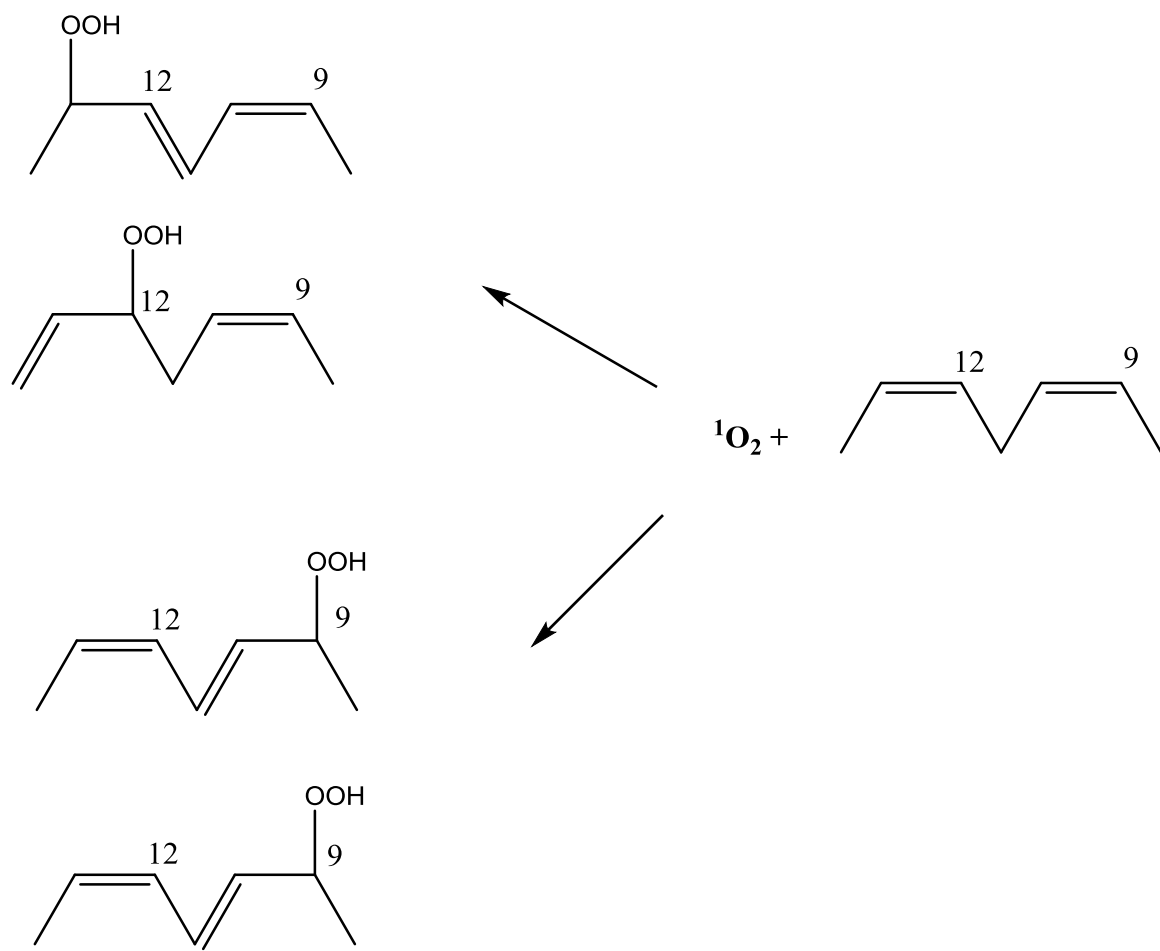


Figure 2.16 Photooxidation mechanism of linoleate (Frankel, 1984).

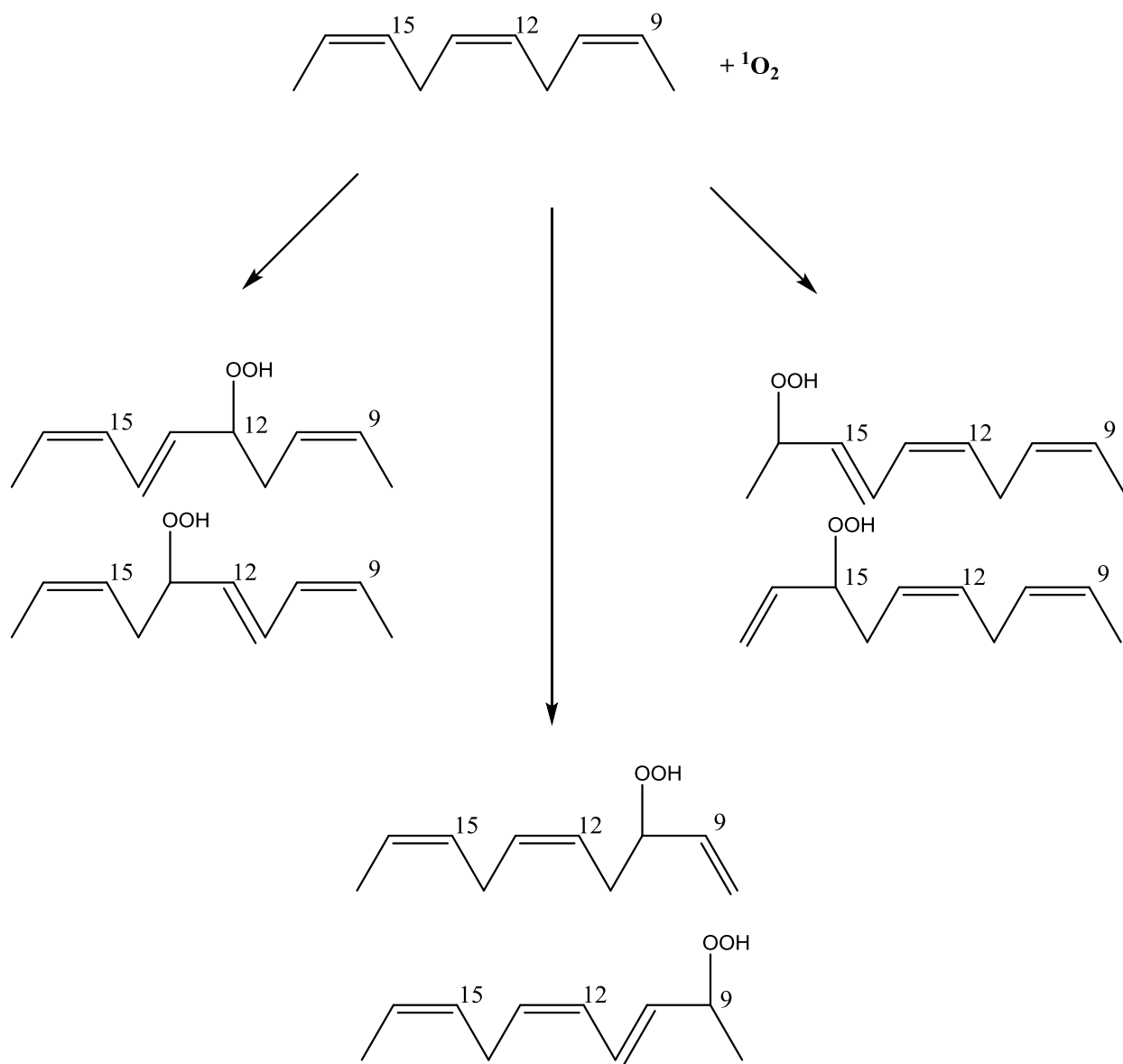


Figure 2.17 Photooxidation mechanism of linolenate (Frankel, 1984).

The rapid photooxidation can be followed by autooxidation which at the end may lead to multiple intermediates and oxidation products which change with time as well as reaction conditions. Thus, photooxidized hydroperoxides may also decompose and produce secondary oxidation products (Frankel, 1984). Photooxidation has a significant effect on oxidative changes in foods as well as biological organisms exposed to solar light and UV radiation.

2.4. Removal of minor components

Several chromatographic techniques have been developed and used to remove minor components from oils. One study used a dry column packed with silicic acid in certain proportions, charcoal, sugar, and celite in order to prepare large amounts of stripped soybean oil (Mistry & Min, 1988). The product (stripped oil) was odourless, colourless, tasteless and free from minor components such as tocopherols, carotenoids, phospholipids, free fatty acids, as well as mono- and diacylglycerols. Later, Baldioli, Servili, Perretti and Montedoro (1996) employed this technique to prepare stripped olive oil. However, this technique is not always effective. Lampi, Hopia, Ekholm and Piironen (1992) used this technique to remove the minor components from rapeseed oil; however, the stripped oil still retained about 60% γ -tocopherol and up to 35% α -tocopherol. Furthermore, Khan and Shahidi (2000) improved this technique by using a column packed with silicic acid and activated charcoal in the middle layer; the oil sample dissolved in the same amount of hexane and was passed through the column. They used a water vacuum in order to save time. This technique improved the removal of minor components from evening primrose, hemp, flax, soybean and olive oils (Khan & Shahidi, 2001; Abuzaytoun & Shahidi, 2006; Lampi et al., 1992). Another study used this

technique to strip soybean oil by using a chromatographic column separation process, but with a lesser amount of two layers of silicic acid (22.5 g) and activated charcoal (5.625 g), and by employing hexane as eluent (Tian, Decker & Goddard, 2013). Li, Wang and Shahidi (2016) further improved this technique with a minor change by using 45 g of activated silicic acid, followed by 45 g of charcoal and 45 g of activated silicic acid, and the samples used were blackberry, black raspberry, and blueberry seed oils. The results proved that stripped seed oils so obtained were devoid of any tocopherol, therefore confirming that this method was quite effective in removing minor components from these tested oils.

2.5. Measurement of lipid oxidation

To evaluate the degree of lipid oxidation, it is very important to choose appropriate methods from among a variety of analytical procedures that are available for the evaluation of edible oils. As far as we know, lipid oxidation products are complex and may include both primary and secondary oxidation products, so there is no standard method that can evaluate the overall oxidative status in all foods. Thus, it is necessary to use more than one method in order to measure both primary and secondary oxidation products. These methods can be categorized into five groups depending on what they measure. These involve the formation of free radicals, loss of initial substances, the absorption of oxygen, as well as determination of primary and secondary oxidation products (Dobarganes & Velasco, 2002). In addition to physical, chemical and sensory techniques, instrumental methods can also be used to measure lipid oxidation parameters and to predict the shelf life of products. According to Wanasundara, Shahidi and Jablonski (1995), sensory analyses are more accurate. However, they are expensive and

time consuming; therefore, they are not the best choice in routine laboratory analysis. Thus, chemical, physical and instrumental methods are commonly used. These methods of analysis include determination of peroxide value, iodometric titration, and spectrometry for conjugated dienes and trienes. Additional methods include headspace oxygen uptake, specific volatile aldehyde measurement, oxidative stability index (OSI), and 2-thiobarbituric acid (TBA) value, among others.

2.5.1. Primary products of lipid oxidation

At the beginning of the process, that is the initiation stage, the primary products of lipid oxidation (hydroperoxides) are produced, but these can decompose very quickly into a number of volatile and non-volatile secondary oxidation products. The rate of hydroperoxide formation exceeds their decomposition; however, the decomposition rate outruns the formation of hydroperoxides at later stages of initiation. Therefore, in order to measure hydroperoxides, one must consider the time factor that can be the indicator of initial stages of hydroperoxide formation (Shahidi & Wanasundara, 2002). Moreover, the measurement of conjugated dienes is simple, fast, requires a very small amount of sample and also does not need any chemical reagent, so it is a desirable method to evaluate primary products of lipid oxidation. The conjugated dienes show an intense absorption from 230 to 235 nm in the ultraviolet (UV) region and the increase in the ultraviolet (UV) absorption reflects the production of primary lipid oxidation products. In general, conjugated dienes show a UV absorption peak at 234 nm, whereas conjugated trienes absorb at 268 nm (Shahidi & Wanasundara, 2002; Shahidi & Zhong, 2015).

Conjugated dienes (CD) have less specificity and sensitivity in comparison with peroxide value (PV) measurements. Furthermore, the results might be influenced by

other compounds and absorb at 234 nm (Shahidi & Wanasundara, 2002). To prevent this, it is necessary to have another spectroscopic method to measure conjugatable oxidation products (COP) since hydroperoxides as well as other decomposition products could be changed to more conjugated chromophores, like conjugated trienes absorbing at 268 and the tetraenes absorbing at 301nm (Gordon, 1990; Shahidi & Wanasundara, 2002).

2.5.2. Secondary products of lipid oxidation

Primary products of oxidation are known to be unstable and decompose into a complex mixture of ketones, aldehydes, alcohols, hydrocarbons, and volatile organic acids, among others. The thiobarbituric acid (TBA) method is frequently used for the assessment of lipid oxidation. This test is used to quantify the concentration of secondary oxidation products, known as TBA reactive substances (TBARS), and to report the results as malondialdehyde (MDA) equivalents. The reaction is shown in Figure 2.18. One molecule of MDA which is a secondary product of lipid oxidation reacts with two molecules of TBA to afford the TBA-MA complex (pink colour) which has absorbance at 532 nm. Other than MDA, the TBA reagent also reacts with alkenal and alkadienals, thus TBARS have been widely used instead of MDA to express TBA values (King et al., 1993).

The disadvantage of the TBA method is that it lacks specificity and sensitivity. In addition, TBA can also react with sugars and oxidized proteins. However, the common edible oils contain little or no sugar or oxidized protein, leading to the conclusion that the TBA test can be used to measure lipid oxidation in foods (Jardine, Antolovich, Prenzler & Robards, 2002).

In addition to measuring the complexes of secondary oxidation products, volatile aldehydes such as propanal, hexanal, and nonanal arise from the oxidation of n-3, n-6 and n-9 fatty acids, respectively. Furthermore, aldehydes such as pentanal, 2-pentenal, butanal, octanal, and 2,4-decadienal, among others, might also be used to evaluate the oxidation of lipids. Oxidized lipid samples are heated to vaporize the volatile components which are the decomposition products of hydroperoxides and then the volatiles in the headspace above the samples are analyzed using gas chromatography (GC). Gas chromatographic analysis of the headspace volatiles is rapid and simple, because of the injection of aliquots of volatile compounds from the headspace of the samples. However, the disadvantage of this method is the difficulty of reaching complete equilibrium with viscous and semi-solid samples. In addition, polyunsaturated lipid samples could decompose during the heating period.

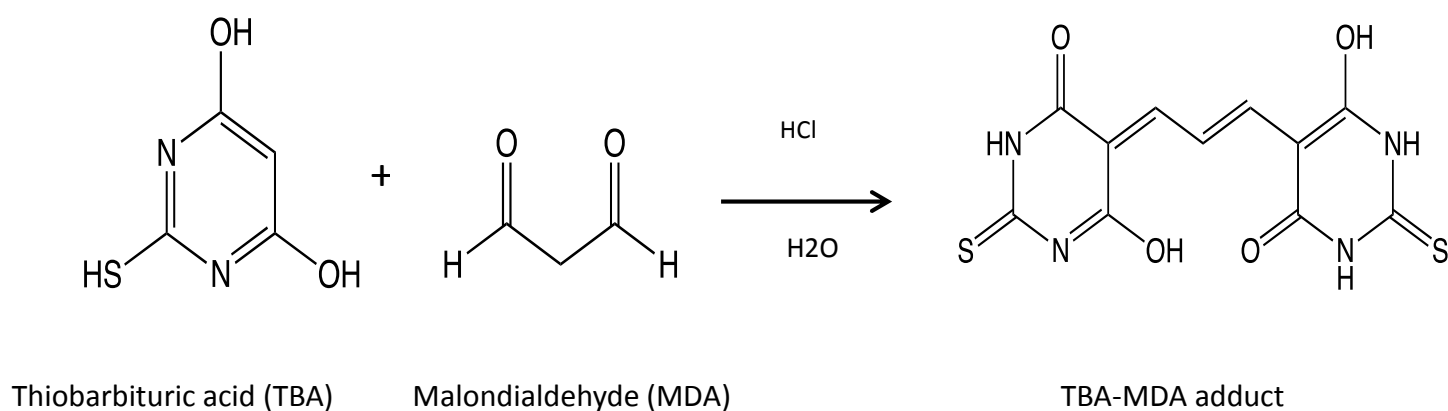


Figure 2.18 The formation of malondialdehyde-thiobarbituric acid chromogen (Shahidi & Wanasundara, 2002).

2.6. Control of lipid oxidation

Food antioxidants can be classified into two categories: natural and synthetic. The oxidation of edible oils and fats can be controlled by their use and/or use of inert gas or vacuum packaging. In general, ideal antioxidants (synthetic or natural) for food applications must meet certain criteria such as safety, effectiveness at low concentrations, maintenance of flavour and colour, resistance to high temperatures experienced during frying and baking as well as availability at low cost (Coppen, 1994).

2.6.1. Synthetic antioxidants

Since 1940's, synthetic antioxidants have been introduced to the food industry (Shahidi & Zhong, 2010). Phenolic antioxidants inhibit lipid oxidation by acting as hydrogen or electron donors and interfere with the free radical chain reaction by preventing their further propagation (Figure 2.19). Synthetic antioxidants like butylated hydroxytoluene (BHT), butylated hydroxyanisole (BHA) and tertiary-butylhydroquinone (TBHQ) are commonly used in the food industry (Figure 2.20). The effectiveness of these antioxidants is due to the stability of their phenoxyl radicals through resonance delocalization (Berger & Hamilton, 1994).

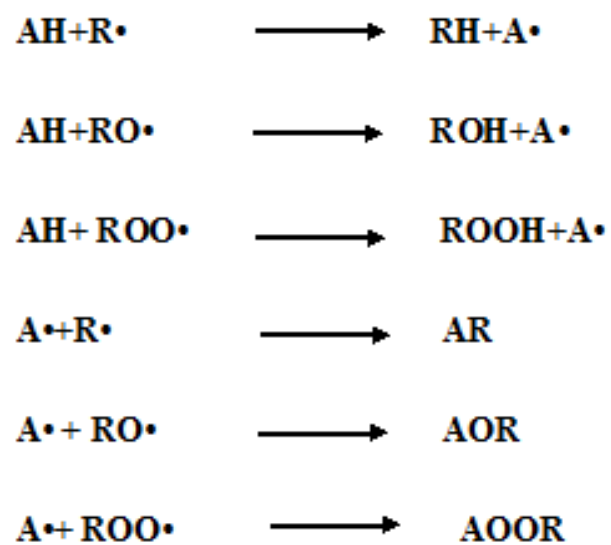
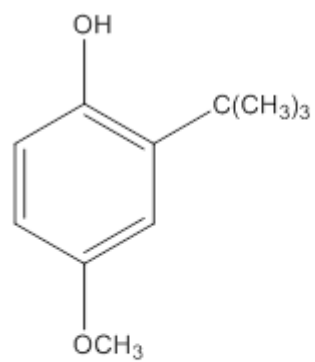
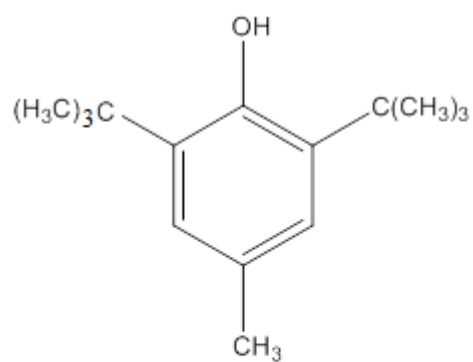


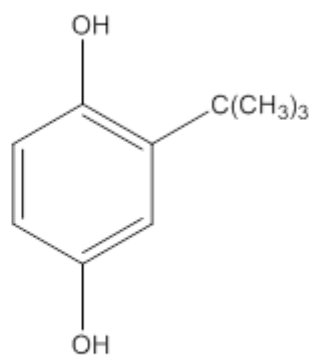
Figure 2.19 Formation of non-radical compounds in the termination reaction of free radicals.



BHA



BHT



TBHQ

Figure 2.20 Chemical structures of common synthetic antioxidants; BHA, BHT and TBHQ (Shahidi & Zhong, 2010).

Both BHA and BHT are soluble in animal fats and vegetable oils but are generally more effective in protecting animal fats. Meanwhile, TBHQ is a powerful antioxidant for vegetable oils (Coppen, 1994). Moreover, nowadays, the use of these antioxidants is limited due to the concern over their potential carcinogenic effects (Shahidi & Zhong, 2010).

2.6.2. Natural antioxidants

Application of natural antioxidants to prevent the oxidation of food is growing rapidly because of the increase in consumer demands and the controversy about the use of synthetic antioxidants due to their potential health concerns (Bruun-Jensen, Skovgaard & Skibsted, 1996). Furthermore, consuming foods rich in natural antioxidants might provide protection and improve the health status of humans (Ramarathnam Osawa, Ochi & Kawakishi, 1995; Wanasundara, Shahidi & Shukla, 1997; Ramadan, Kroh & Mörsel, 2003).

This protection could be explained by the ability of these antioxidants to scavenge free radicals that are responsible for the oxidative damage to lipids and proteins (Aruoma, 1998). Vegetables, fruits, spices, cereals, herbs, grains, seeds, oils, and teas are important sources of plant-derived antioxidants, especially phenolic compounds including tocopherols. In addition, there is an interest in the antioxidants originating from marine sources such as fish/shellfish, algae, and marine bacteria (Kim, Kim, Byun, Nam, Joo & Shahidi, 2001; Athukorala et al., 2003; Shahidi & Zhong 2010). As noted earlier, a variety of antioxidants have been characterized, among which tocopherols, carotenoids, pigments, and polyphenols that are present in each oil and contribute to its oxidative stability could be

mentioned (Shahidi, Wanasundara & Amarowicz, 1994; Shahidi & Shukla, 1996; Shahidi, Metusalach & Brown, 1998; Metusalach, Brown & Shahidi, 1999; Byrd, 2001; Naczk & Shahidi, 2004; Shahidi & Naczk, 2004; Alamed, Chaiyasit, McClements & Decker, 2009; Shahidi & Zhong, 2010, 2011; Smith & Hong-Shum, 2011; Ixtaina et al., 2012; Shahidi & Ambigaipalan, 2015; Akoh, 2017).

CHAPTER 3

MATERIALS AND METHODS

3.1. Materials

The samples of camelina (*Camelina sativa*) seeds were obtained via Professor Christopher Parrish, Memorial University, St. John's, NL, Canada. Chia seeds (*Salvia hispanica*) used in this work were bought from Costco Wholesale, St. John's, NL, Canada. Meanwhile, sophia seeds were a product of Daghdaghabad, near Hamedan city in Iran, and purchased from the Tavazo store in Toronto, ON, Canada. The compound 2-thiobarbituric acid, silicic acid powder (mesh size: 100-200, acid-wash), activated charcoal, and standards of tocopherols were bought from Sigma-Aldrich (Mississauga, ON, Canada). Standard fatty acid methyl esters (FAMES; GLC-461) were purchased from Nu-Check (Elysian, MN, USA). Compressed air was purchased from Canadian Liquid Air Ltd. (St. John's, NL, Canada). Methanol, hexane, acetonitrile, ethanol, sulphuric acid, 1-butanol, isopropanol, isooctane and all other chemicals were acquired from Fisher Scientific Co. (Nepean, ON, Canada), and were used without further purification. All solvents were ACS grade or better, unless otherwise specified.

3.2. Methods

2.2.1. Fat Extraction

Hexane Extraction. The oils from crushed camelina, chia, and sophia seeds were extracted according to the method described by Miraliakbari and Shahidi (2008) with minor modifications. Fifty grams of seed sample were ground into a powder, then combined with 250 mL of hexanes, followed by homogenization using a blender (Waring Blender model 51BL30, WARING commercial, Phoenix, AZ, USA) for 2 min. The resulting mixture was filtered twice through Whatman number 1 filter paper with a Buchner funnel. The residue was re-extracted five times, and the solvent was removed from a portion of the extract using a rotary evaporator at 40°C. The oil was weighed and transferred into 500 mL sample vials, flushed with nitrogen gas, and stored at -80°C until use.

3.2.2. The removal of minor components from camelina, chia, and sophia seed oils

3.2.2.1. Column chromatographic method

The seed oils from the camelina, chia, and sophia were stripped of their minor constituents according to the technique outlined by Abuzaytoun and Shahidi (2006). It is important to note that minor adjustments were made to the technique to customize it to this specific experiment. This technique employed the use of a chromatographic column which had a length of 40 cm and an internal diameter of 3.4 cm.

The chromatographic column was filled consecutively with various components as follows; the first layer consisted of 45 g of activated silicic acid, the next layer 45 g of charcoal, and the last layer was filled with an additional 45 g of activated silicic acid. It is crucial to point out that 100 g of silicic acid was activated by washing three times using a total of 3L of distilled water before it was introduced to the solvent. After each treatment, the silicic acid was allowed an average of 30 min for settling before the liquid was discarded.

Approximately 60 g of hexane were used to dilute 60 g of oil. The mixture was then passed through the chromatographic column, followed by another 200 mL of hexane for elution. The solvent was then removed from the stripped oil through evaporation under vacuum at 50°C. Afterwards, the column-stripped oils were placed into 10 mL glass vials. Similarly, nitrogen was used again to flush any possible remaining solvent in the oil and then stored at -80°C until use which was less than one month. The conditions and duration of the storage of the samples were maintained prior to subsequent experiments.

3.2.2.2. A Novel and simplified stripping method using stationary phase (silicic acid/charcoal) material

Stripped camelina, chia, and sophia oil were prepared according to a simplified stripping method (stationary phase). The oil (60 g) with hexane (60 mL) was stirred with activated silicic acid (90 g) and charcoal (45 g) for 2 h under nitrogen (atmosphere). This operation was accomplished in the fume hood at 27.5°C, followed by suction filtration. In addition, 200 mL of hexane were used to wash the materials during the filtration. The

solvent was removed as done previously using a rotary evaporator at 40°C, followed by nitrogen flushing; stripped oils were transferred into 10 mL glass vials which were then stored at -80°C prior to use.

3.2.3. Analysis of camelina, chia and sophia seed oils

3.2.3.1. Fatty acid composition

This method involved the analysis of fatty acid compositions of the column-stripped, simplified stripping method (stationary phase), and non-stripped camelina, chia, and sophia seed oils by transmethylation. The composition of the fatty acids of the samples were evaluated by using gas chromatography-flame ionization detection (GC-FID).

The transmethylation reagent (2.0 mL), consisting of freshly prepared 6% sulphuric acid in methanol containing about 15 mg of hydroquinone as an antioxidant was used as specified by Wanasundara and Shahidi (1997). The results of the experiment were expressed as the weight percentage associated with each fatty acid. The mixture was incubated for 24 h at 60°C and subsequently cooled to room temperature. Distilled water (1.0 mL) was then added to the mixture. The FAMES were extracted three times, each with 1.5 mL of HPLC grade hexane. The combined hexane layers were subsequently transferred to test tubes and washed twice times with 1.0 mL of distilled water. The hexane was subsequently evaporated under nitrogen.

The positional distribution and composition of fatty acids associated with the products were established after they were converted to the corresponding methyl esters. Analysis of the fatty acid methyl esters (FAMES) was performed using a Hewlett-

Packard 5890 series II gas chromatograph. The Hewlett-Packard 5890 series II gas chromatograph was fitted with a Supelcowax-10 column. The Supelcowax-10 was 30 m long with a diameter of 0.25 mm and a film thickness of 0.25 μm . The identification process of FAMES was performed through comparison of their retention time with those of a standard mixture, the standard fatty acid methyl esters (FAMES; GLC-461) were procured from Nu-Check (Elysian, MN, USA).

3.2.3.2. Triacylglycerol profiles

The method outlined by Lisa and Holcapek (2008) was used for the determination of TAG in the tested seed oils. This method was successful due to the use of the high-performance liquid chromatography-photodiode array detection-atmospheric pressure chemical ionization-mass spectrometry (HPLC-DAD-APCI-MS) (Li, Wang & Shahidi, 2016). Similarly, this method was subjected to minor alterations to customize it for this test. Acetonitrile/isopropanol (1:1, v/v) mixture was used to dissolve the oils in order to obtain a 3% (w/v) solution.

The chromatographic system consisted of an 1100 Series Agilent Technologies LC-MSD system equipped with a diode array detector (DAD) coupled to a mass spectrometer equipped with an atmospheric pressure chemical ionization (APCI) interface (Agilent, Palo Alto, CA, USA). The separation was performed on C-18 columns (4.6mm \times 250mm, Sigma) using a gradient elution with acetonitrile (A) and 2-propanol (B). Consequently, the system used was as follows: 0 min, 100% A; 106 min, 31% A; 120 min, 100% A. The UV at 215 nm and positive-ion APCI-MS were coupled in a series (Li, Wang & Shahidi, 2016). MS parameters were as follows: vaporizer

temperature, 400°C; drying gas temperature, 350°C; gas flow (N₂), 3.0 L/min; nebulizer pressure, 60 psi.

3.2.3.3. Measurement of minor components

3.2.3.3.1. Measurement of pigments (chlorophylls and carotenoids)

Measurement of the pigments in the non-stripped and stripped oil samples was carried out by following the absorbance at 430-460 nm for carotenoids and 550-710 nm for both chlorophylls and their by-products. Hexane (equal volume) was mixed with the oil samples. The mixture was then placed into quartz cuvettes (Abuzaytoun & Shahidi, 2006), and the absorbance read using a 8453A UV-Visible spectrophotometer (Agilent Technologies, Palo Alto, CA, USA) and recording the absorption spectra between 430 and 710 nm.

3.2.3.3.2. Determination of tocopherols

To determine the content of tocopherols in camelina, chia, and sophia seed oils, the oil samples were prepared by dissolving 0.1 g of oil in 2 mL of methanol/acetonitrile/isopropanol (41:59:300, v/v/v). Tocopherol standards were prepared by dissolving a known amount of tocopherol mixture that consisted of 4 tocopherols in the same solvent followed by serial dilution. Before the HPLC analysis, the samples, as well as standards, were filtered by using a 0.45 µm Whatman syringe-filter.

Tocopherol contents in the prepared samples and standards were determined by a reversed phase HPLC-MS, using an Agilent 1100 HPLC unit (Agilent Technologies, Palo Alto, CA, USA), equipped with a UV–diode array detector (UV-DAD). Separation was achieved on a C-18 column (4.6 mm × 250 mm coupled with a guard column, Agilent) by gradient elution with a methanol/acetonitrile/isopropanol mobile phase at a flow of 0.8 mL/min; the fractions were detected at 295 nm. Mobile phase started with methanol/acetonitrile/isopropanol (41:59:0, v/v/v) and was maintained for 15 min.

The mobile phase was gradually changed to methanol/acetonitrile/isopropanol (16.5:23.5:60, v/v/v) from 15 to 25 min, and then changed to 100% of isopropanol from 25 to 35 min. The mobile phase was changed to its initial state, methanol/acetonitrile/isopropanol (41:59:0, v/v/v) in 5 min, and was kept there for 10 min. LC flow was analyzed on-line by a mass spectrometric detector system (LC-MSD-Trap-SL, Agilent, Palo Alto, CA, USA) using positive ion APCI (atmospheric pressure chemical ionization). The operating conditions were 121 V for the fragments with a drying temperature of 350°C, APCI temperature of 400°C, nebulizer pressure of 60 psi, drying gas flow of 7 l/min for each tocopherol standard and sample (50 µL) was injected. Tocopherols were detected at 295 nm using a UV detector and identified by comparing their retention times with those of known tocopherol standards. A standard curve was constructed for each tocopherol homologue. Tocopherol concentrations in the samples were calculated using the standard curve and expressed as mg/kg of oil.

3.2.4. Oxidative stability tests of the original seed oils and their stripped counterparts under accelerated oxidation conditions using a Schaal oven and fluorescent lighting

An 8453A UV-vis spectrophotometer played a significant role in measuring the concentration of the subsequent coloured complex at 532 nm. Afterward, a standard line, prepared using 1,1,3,3-tetramethoxypropane, was used to report the values of thiobarbituric acid reactive substances (TBARS) (Li, Wang & Shahidi, 2016).

Two separate conditions were used to examine the oxidative stability of non-stripped and stripped seed oils. The samples were examined under fluorescent light and Schaal oven conditions. The Schaal oven conditions, at 60⁰C for 7 days, were used to determine the oxidative stability associated with the stripped seed oils (Li, Wang & Shahidi, 2016). It is worth mentioning that every 24 hours of storage under Schaal oven conditions is similar to one-month of storage at ambient temperatures (Evans, List, Moser & Cowan, 1973). After 1, 3, 5, and 7 days, the oil samples were removed from the oven. The non-stripped samples of seed oils, which were examined under fluorescent radiation (photo-oxidation), were stored in a rectangular polyethylene box. The box measured 35 cm wide, 70 cm long and 25 cm high. It was fitted with two 40 W cool white fluorescent lights. The white fluorescent lights were positioned roughly 10 cm above the exterior of the oil containers.

The level of the fluorescent radiation was kept at 2650 Lux while the temperature within the container was maintained at 28.5±1°C. The oil samples were removed from the lightbox after 1, 3, 6, 12, 24, 48, and 72 h, flushed with nitrogen, and parafilm was

then wrapped around the caps of the sample containers. The containers were then kept at -80°C until they were tested for oxidative stability. These tests were carried out within one month of collection to insure the viability of the samples (Li, Wang & Shahidi, 2016).

The steps outlined by the International Union of Pure and Applied Chemistry (IUPAC) were followed to determine the conjugated dienes (CD) of the oil samples. The following equation was used to compute the value of the conjugated dienes (CD) associated with the oil samples.

$$CD = A / (cd)$$

The absorbance of the solution at 234 nm is represented by A, while the concentration of the solution in grams per 100 mL is represented by C. On the other hand, the length of the cell was represented by d in 1 cm. The steps outlined by the American Oil Chemists' Society (AOCS, 1990) were used to determine the contents of thiobarbituric acid reactive substances (TBARS) (Li, Wang & Shahidi, 2016).

3.2.4.1. Determination of lipid oxidation

The oxidative stability of the stripped and non-stripped oils was evaluated by measuring conjugated dienes (CD) for primary and TBARS for secondary oxidation products.

3.2.4.1.1. Determination of conjugated dienes (CD)

The IUPAC (1987) procedure was used to determine the conjugated dienes (CD) contents of the oil samples (Abuzaytoun & Shahidi, 2006). About 0.03g of oil was

weighed and transferred to a 25mL volumetric flask. The weighted oil samples were then dissolved in isooctane (2,2,4-trimethylpentane) (Abuzaytoun & Shahidi, 2006). The same solvent (isooctane) was added to the mixture to raise to volume to the correct level. After raising/filling the mixture up to the correct mark in the volumetric flasks, the contents were subjected to a thorough mixing.

A Hewlett-Packard 8456A diode array spectrophotometer was used to read the absorbance of the contents at 234 nm within a 10-mm Hellma quartz cell (Abuzaytoun & Shahidi, 2006). Pure isooctane was utilized as the blank. The following equation was used to compute the values of conjugated dienes (CD) as given below.

$$CD= A/ (C \times d)$$

where C represents the concentration of the solution in grams per 100 mL solution (g/100mL), while A represents the absorbance of the solution at 234 nm and the length of the cell is represented by d (in cm) (Abuzaytoun & Shahidi, 2006).

3.2.4.1.2. Determination of 2-thiobarbituric acid-reactive substances (TBARS)

The steps outlined by the American Oil Chemists' Society (AOCS, 1990) were carefully followed to analyze the oil samples (0.05 -0.20 g) for their contents of 2-thiobarbituric acid-reactive substances (TBARS) (Abuzaytoun & Shahidi, 2006). The weights of the oil samples were precisely determined, and then the samples were transferred to 25 mL volumetric flasks. A small volume of 1-butanol was used to dissolve the oil samples in the volumetric flasks. The same solvent (1-butanol) was used to bring the sample solution levels up to the mark (Abuzaytoun & Shahidi, 2006).

A percentage of the mixture (5.0 mL) was placed into a dry test tube. A combination of 5 ml of a solution of 200 mg 2-TBA dissolved in 100 mL 1-butanol was used as a fresh 2-TBA reagent (Abuzaytoun & Shahidi, 2006). The reagent was then added to the previously prepared mixture in a dry test tube. A water bath at 95°C was used to heat the contents of the dry test tube for two hours followed by mixing (Abuzaytoun & Shahidi, 2006).

Afterward, a Hewlett-Packard 8452A diode array spectrophotometer was used to read the absorbance of the resultant coloured complex at 532 nm. The TBARS values were determined by multiplying the absorbance readings by a factor of 0.415, obtained from a standard curve that used 1,1,3,3- tetramethoxyprpane as a precursor of malondialdehyde (MDA) (Abuzaytoun & Shahidi, 2006).

3.2.5. Statistical analysis and data interpretation

All the experiments were conducted in triplicate in each sample. ANOVA, which is a single-way evaluation of variance, together with Tukey's standardized test, conducted at $p < 0.05$ through the use of Minitab statistical software, were used for the statistical treatment of the data. It should be noted that the version of the Minitab statistical software used was version 14 (attributed to Minitab Inc, State College, PA, USA). Both Tukey's standardized test and ANOVA were utilized to evaluate the significance of difference that exists among the values. The final results were reported as means \pm SD. The second version of SigmaStat for Windows was used to examine normality (Abuzaytoun & Shahidi, 2006). For the recovery of the column stripping and stripped by stationary phase (2 h), an independent sample t-test was employed.

CHAPTER 4

RESULTS AND DISCUSSION

4.1. Removal of minor components from oils using column stripping and stripping by stationary phase (silicic acid/charcoal; 2 h) methods

In order to evaluate the antioxidant/ pro-oxidant effects of minor components of camelina, chia, and sophia oils, they were removed by two methods; the column stripping method and a newly developed method called stationary phase (silicic acid/charcoal) method. The column chromatographic method was applied by passing the oil (60 g of oil dissolved in 60 mL hexane) through a column packed with activated silicic acid (first layer 45 g and last layer 45 g) and activated charcoal (45 g middle layer). The simplified stripping method (stationary phase), on the other hand, was conducted via dissolving the test oil (60 g) in hexane (60 mL) was stirred with activated silicic acid (90 g) and charcoal (45 g) for 2 h under nitrogen, followed by suction filtration. The solvent was then removed using a rotary evaporator at 40 °C.

The oil yield from the column stripping method was 65.26-67.92%, which was higher than that of the stationary phase (silicic acid/charcoal; 2 h) (62.31-65.24%; Table 4.1). The column chromatographic method required a longer processing time; however,

it afforded a high oil yield. The column chromatographic method and oil stripped by the stationary phase (2 h) method gave similar results in terms of minor component removal, such as tocopherols and pigments. Therefore, further experiments in this study were mostly conducted with both column stripped and stripped by stationary phase (2 h) and results were compared with non-stripped oils.

Table 4. 1 Recovery (g of resultant oil/ 100 g of oil) of stripped camelina, chia, and sophia seed oils by the column stripping and stripped by stationary phase (2 h)¹.

Seed oil sample	Column stripped	Stripped by stationary phase (2h)
Camelina	65.26±0.132 ^a	62.31±0.203 ^b
Chia	71.65±0.244 ^a	67.43±0.432 ^b
Sophia	67.92±0.313 ^a	65.24±0.311 ^b

¹Values are mean value of triplicate determination ± standard deviation; Values in the same row with different superscripts are significantly different (p<0.05).

4.1. Characterization of camelina, chia and sophia seed oils

The characteristics of edible oils depended on several factors, such as carbon chain length of the fatty acids, degree of unsaturation, presence of geometric isomers of fatty acids, their positional distribution on the glycerol backbone, and the molecular configuration of triacylglycerols (TAGs). Therefore, this study was conducted to evaluate camelina chia, and sophia seed oils by analyzing their aforementioned compositional characteristics.

4.1.1. Fatty acid composition

Major fatty acids such as palmitic (16:0), stearic (18:0), oleic (18:1), linoleic (18:2n-6), and α -linolenic (18:3n-3) acids were detected in the non-stripped and column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oils (Table 4.2). All seed oils tested contained quite a high amount of total polyunsaturated fatty acids (PUFAs), ranging from 52.7 to 78.4%. Chia seed oil had the highest amount of PUFAs (78.4%) and the lowest content of saturated fatty acids (10.8%), while camelina seed oil contained the highest level of monounsaturated (31.8%); α -linolenic (18:3) acid was the most prevalent fatty acid in all tested seed oils, contributing 36.2-61.2% to the total content of fatty acids. The highest level of this fatty acid was observed in chia seed oils including non-stripped, column stripped and stripped oil by stationary phase (2 h). All tested seed oils also contained significant amounts of linoleic (18:2) acid, the level of this fatty acid was about 14.3, 15.3, and 16.7% in sophia, camelina, and chia, respectively. Figure 4. 1 provides a clear image of major fatty acids found in all tested oils.

The results presented in Table 4.2 for camelina seed oils correspond favourably with those reported in the literature (Leonard, 1998; Zuhr & Matthaus, 2002; Eidhin et al., 2003; Abramovic & Abram, 2005; Moser & Vaughn, 2010; Hrastar, Cheong, Xu, Miller & Kosir, 2011). Thus, camelina seed oil contained roughly 14.3% of oleic acid, 18.8% linoleic, and 36.8% α -linolenic acids. Chia seed oil contained 6.8% oleic acid, 18.6% linoleic acid, and 64.6% α -linolenic acid (Ayerza, 1995; Ayerza & Coates, 2004; Chicco et al., 2009; Ixtaina et al., 2012; da Silva Marineli et al., 2014).

Table 4. 2 Major of fatty acid composition (area percent) of non-stripped and column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oil^{1,2}.

FA (%)	Camelina oil				Sophia oil		Chia oil		
	NCO	CS-CO	SS-CO	NSO	CS-SO	SS-SO	NChO	CS-ChO	SS-ChO
16:0	5.8±0.227 ^a	5.5±0.013 ^a	5.3±0.111 ^a	4.2±0.032 ^a	4.1±0.021 ^a	4.3±0.104 ^a	6.7±0.101 ^a	6.5±0.132 ^a	6.6±0.222 ^a
18:0	2. 6±0.115 ^a	2.7±0.005 ^a	2.9±0.104 ^a	2.2±0.026 ^a	2.5±0.233 ^a	2.3±0.132 ^a	3.2±0.111 ^a	3.5±0.044 ^a	3.4±0.104 ^a
18:1	13.9±0.622 ^a	13.8±0.515 ^a	13.6±0.343 ^a	9.4±0.032 ^a	9.3±0.212 ^a	9.4±0.204 ^a	6.0±0.104 ^a	6.0±0.053 ^a	6.3±0.108 ^a
18:2	14.9±0.343 ^a	15.1±0.703 ^a	15.3±0.401 ^a	14.3±0.171 ^a	14.1±0.221 ^a	14.3±0.202 ^a	16.6±0.023 ^a	16.7±0.023 ^a	16.4±0.344 ^a
18:3	36.1±0.133 ^a	36.2±0.210 ^a	35.9±0.123 ^a	46.2±0.644 ^a	45.9±0.903 ^a	46.1±0.630 ^a	61.1±0.944 ^a	61.2±0.711 ^a	61.0±0.915 ^a
20:0	2.1±0.147 ^a	2.3±0.023 ^a	2.1±0.120 ^a	2.6±0.101 ^a	2.7±0.145 ^a	2.5±0.142 ^a	0.8±0.105 ^a	0.8±0.022 ^a	0.8±0.043 ^a
20:1	15.8±0.362 ^a	15.5±0.617 ^a	15.7±0.233 ^a	8.6±0.121 ^a	8.2±0.255 ^a	8.4±0.152 ^a	0.8±0.044 ^a	0.9±0.040 ^a	0.8±0.033 ^a
20:2	1.7±0.023 ^a	1.6±0.022 ^a	1.9±0.012 ^a	2.0±0.032 ^a	2.1±0.032 ^a	2.2±0.132 ^a	0. 6±0.011 ^a	0.5±0.033 ^a	0.6±0.042 ^a
22:1	2.1±0.011 ^a	1.9±0.101 ^a	2.0±0.102 ^a	9.0±0.144 ^a	9.2±0.201 ^a	9.1±0.301 ^a	-	-	-
ΣSFA	10.5	10.5	10.3	8.9	9.4	9.1	10.8	10.3	10.8
ΣMUFA	31.8	31.4	31.3	27.0	26.7	26.9	6.9	6.8	7.1
ΣPUFA	52.7	52.9	53.1	62.5	62.2	62.6	78.3	78.4	78.0

¹Values are mean of triplicate determination ± standard deviation. Values in the same row for each oil with different superscripts are significantly different (P<0.05); ²Abbreviations: NCO, non-stripped camelina seed oil; CS-CO, column-stripped camelina seed oil; SS-CO, stripped by stationary phase (2 h) of camelina seed oil; NSO, non-stripped Sophia seed oil; CS-SO, column-stripped sophia seed oil; and SS-SO, stripped by stationary phase (2h) of sophia seed oil; NCh, non-stripped chia seed oil; CS-Ch, column-stripped chia seed oil; and SS-Ch, stripped by stationary phase (2 h) of chia seed oil.

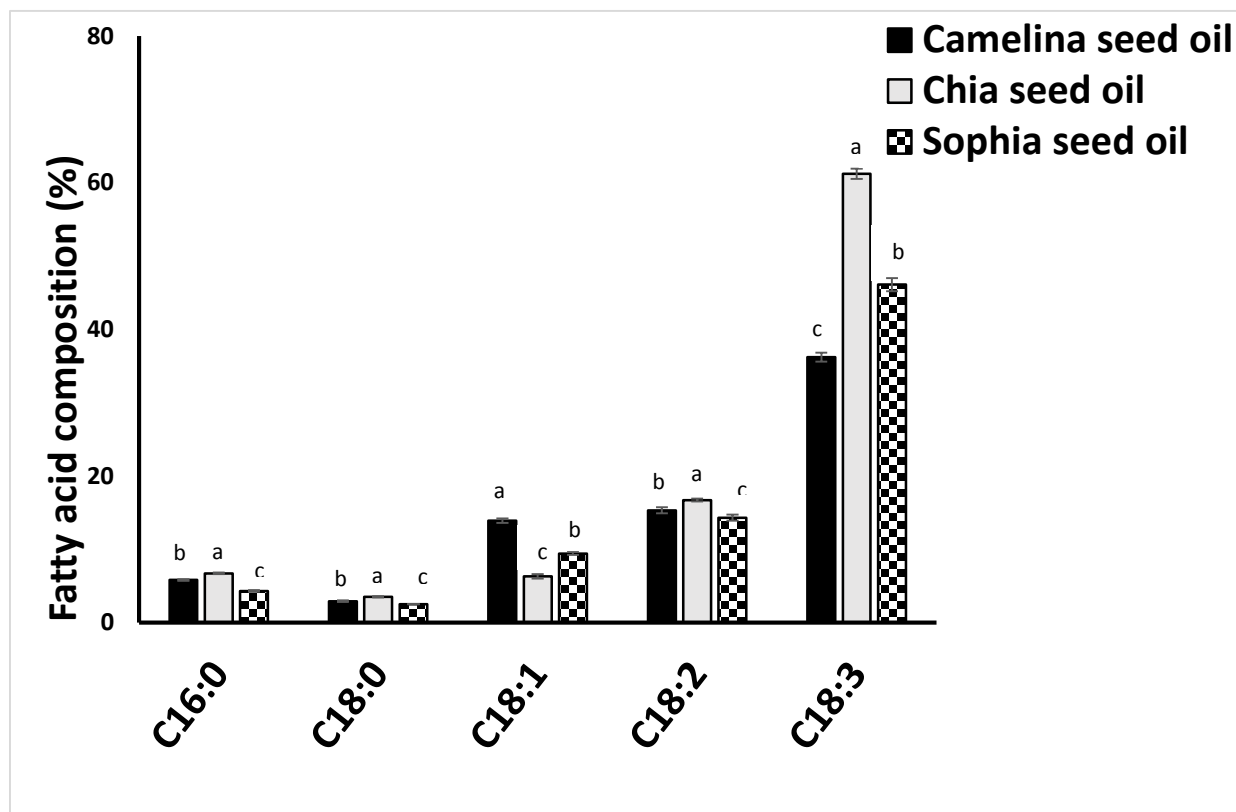


Figure 4. 1 Graphic representation of major fatty acid composition of non-stripped camelina, chia, and sophia seed oils as presented in Table 4. 2.

Sophia seed oil contained 10.2% oleic acid, 16.3% linoleic acid, and 42.1% α -linolenic acid (Dolya et al., 1973; Bekker et al., 2005; Guan et al., 2007; Maršalkienė et al., 2009; Ahmadi & Shahmir, 2016). The small variations between fatty acid profiles presented in this study as well as those reported in the literature may originate from existing differences in the source, and storage conditions, among other possible reasons. For example, differences in the composition of the fatty acids in chia seed oil might be due to environmental conditions, like temperature, and soil type, among others. In particular, Ayerza (1995) reported that the concentration of fatty acids as well as the oil content are influenced by growing location; however, another study found that chia seeds which grew in different ecosystems in South America showed a significant difference in their oil contents, peroxide value, and fatty acid composition (Ayerza & Coates 2004). Examination of the fatty acid profiles for all oils indicated that their fatty acid composition remained unchanged before and after stripping and removing the minor components by either column stripping or by using the stationary phase material (2 h).

4.1.2. Triacylglycerol composition

As mentioned earlier (Chapter 1), triacylglycerols (TAGs) are the most abundant constituents accounting for more than 95% of the edible oils. Therefore, analysis of triacylglycerol species in oils is necessary in order to understand their physical and chemical features, as fatty acid composition can only show a general view of the oxidative stability and nutritional quality of oils.

The triacylglycerols compositions of camelina, chia, and sophia seed oils were determined by using high performance liquid chromatography - diode array detection-atmospheric pressure chemical ionization-mass spectrometry (HPLC-DAD-APCI-MS). TAG composition of the tested oils is presented in Table 4.3. The most prevalent TAGs detected were

trilinolenin (LnLnLn), followed by linolenoyl-linoleoyl-linolenoylglycerol (LnLLn), linolenoyl-linolenoyl-palmitoylglycerol (LnLnP), linoleoyl-linoleoyl-linolenoylglycerol (LLLn), and linolenoyl-oleoyl-linolenoylglycerol (LnOLn). It is significant to state that the triacylglycerol profile of camelina, chia, and sophia has not previously been reported in the literature. Furthermore, no γ -linolenic acid was found in any of the tested oils, therefore Ln in Table 4.3 and elsewhere represents only α -linolenic acid.

The results in Table 4.3 are in agreement with those of fatty acid composition, since the primary fatty acids present in TAG species were also α -linolenic acid, linoleic acid, and oleic acid. The positional distribution of fatty acids within the TAG molecules was determined by mass spectrometry according to the ratio of the fragment ions.

Table 4. 3 Triacylglycerol (TAG) composition (relative concentration percentage) of non-stripped camelina, sophia and chia seed oils^{1,2,3}

TAG	RT	(M+H) MW	NCO	NSO	NChO
LnLnLn	45.45	873	5.08±0.041 ^c	8.84±1.052 ^b	33.51±2.121 ^a
LnLLn	52.61	875	7.27±0.092 ^c	8.58±1.114 ^b	20.25±1.751 ^a
LLLn	59.72	877	5.72±0.033 ^b	4.73±0.101 ^c	6.25±0.042 ^a
LnOLn	61.06	877	5.87±0.075 ^b	5.79±0.045 ^b	6.48±0.051 ^a
LnLnP	62.57	851	3.36±0.014 ^c	4.92±0.103 ^b	9.55±0.141 ^a
LLL	64.67	879	2.41±0.055 ^a	1.26±0.011 ^b	nd
LnLMo	65.4	865	nd	13.97±1.211 ^a	0.28±0.009 ^b
LLLn	66.93	879	1.39±0.016 ^a	nd	0.91±0.014 ^b
OLLn	67.49	879	3.81±0.022 ^a	3.36±0.033 ^b	3.35±0.034 ^b
LnLP	69.5	853	3.16±0.041 ^b	nd	4.30±0.104 ^a
SLnLn	70.73	879	11.39±0.120 ^a	1.61±0.044 ^c	4.34±0.072 ^b
C20:2LL	72.37	907	10.22±0.130 ^b	12.19±1.104 ^a	nd
OLL	74.81	881	5.50±0.081 ^a	2.22±0.020 ^b	0.54±0.010 ^c
OLnO	75.13	881	3.30±0.051	nd	nd
LLP	75.49	855	nd	2.12±0.010 ^a	1.83±0.021 ^a
LnOP	76.12	855	1.5±0.012	nd	nd
SLLn	77.37	881	nd	nd	2.79±0.041
PLnP	79.39	829	nd	nd	0.64±0.034
GLL	79.85	909	3.31±0.020 ^b	8.69±0.11 ^a	nd
OLO	83.94	883	6.38±0.171 ^a	2.77±0.07 ^b	0.51±0.012 ^c
OOPo	84	857	nd	0.81±0.023	nd
SLL	85.04	883	nd	0.45±0.042 ^a	0.68±0.012 ^a
ALLn	85.8	909	4.79±0.031	nd	nd
OLP	85.95	857	nd	nd	0.22±0.005
GOLa	86.73	831	nd	nd	0.61±0.021
SOLn	87	883	1.05±0.005 ^a	1.07±0.051 ^a	nd
BLLn	87.7	937	3.63±0.051 ^a	3.14±0.072 ^b	nd
SLnS	88.5	885	3.19±0.012 ^a	2.17±0.043 ^b	nd
PPP	88.7	807	nd	0.31±0.013	nd
LgLL	91.31	967	nd	nd	0.19±0.009
BLD	93.65	941	nd	1.59±0.034 ^a	0.19±0.007 ^b
LgLO	100.5	969	nd	0.32±0.011	nd
C23:0OO	103	957	2.43±0.053 ^a	1.65±0.021 ^b	nd
LgOO	105	971	0.621	1.033	Nd
Total			95.38	93.54	97.42

¹Values are mean of triplicate determination ± standard deviation. ²Values in the same row with different superscripts are significantly different (p<0.05). ³Abbreviations: NCO, non-stripped camelina seed oil; NSO, non-stripped sophia seed oil; NCh, non-stripped chia seed oil; and nd, not detected.

The oxidative stability of the oil depends on TAG composition (Neff, Mounts & Rinsch, 1997). Neff, List, and Byrdwell (1999) as well as Bakowska-Barczak, Schieber and Kolodziejczyk (2009) reported that the TAG composition affects the taste and texture quality of products, because they could affect functional properties of foods into which the oil is incorporated, such as solid fat index, melting point range, and crystal structure. Triacylglycerol (TAG) types such as LnLP, LLLn, LLP, LLS, LnLO, and PLP decreased oil oxidative stability, whereas LOO, LLO, POO, LOP, LOS, and OOO increased its stability (Neff Mounts, Rinsch & Konishi, 1993; Neff, Mounts, Rinsch, Konishi & El-Agaimy, 1994). Therefore, the results of this study (Table 4.3) demonstrate that all tested seed oils are rich in LnLnLn, LnLLn, LLLn, LnOLn and LnLnP, which are more susceptible to oxidative deterioration than oils rich in oleic acid, such as mango seed kernel oil (Lakshminarayana, Rao & Ramalingaswamy, 1983; Ali, Gafur, Rahman & Ahmed, 1985; Bakowska-Barczak et al., 2009).

4.2. Assessment of minor components in camelina, chia, and sophia seed oils

4.2.1. Assessment of tocopherols in the original, column stripped, and stripped by stationary phase (silicic acid/charcoal; 2 h) of camelina, chia and sophia seed oils

The concentration of tocopherols in camelina, chia, and sophia seed oils and their stripped counterparts using column stripping and stripped by stationary phase (2 h) is shown in Table 4.4. The non-stripped camelina, chia, and sophia seed oils differed in their α -, β/γ -, δ -, and total tocopherols. The total tocopherols in camelina, chia, and sophia seed oils were 1262.54, 341.06, and 1150.93 mg/kg of oil, respectively. The sophia seed oil had the lowest α -tocopherol content in trace amounts (<0.02) but was very rich in β/γ -tocopherol (977.52 mg/kg of oil). The non-stripped camelina seed oil contained the highest concentration of α -tocopherol (114.73 mg/kg of oil), δ -tocopherol (470.41 mg/kg of oil), as well as total tocopherols (1262.54 mg/kg of oil) which was much higher than the other tested seed oils (Table 4.4). However, non-stripped chia seed oil exhibited the lowest concentration of total tocopherols in all samples of oil of α -tocopherol compared to sophia seed oil.

The results presented in Table 4.4 for the total tocopherols of camelina seed oil did not correspond favourably with those reported in the literature (Eidhin, Burke & O'Beirne, 2003). Camelina seed oil contained about 1262.54 mg/kg tocopherols in this study, which is higher than that reported in the literature (789-821 mg/kg g). In another study, Zubr and Matthaus (2002) found that the content of γ -tocopherol in camelina seed oil predominated (651-922 mg/kg) which is similar to that found in this study (677 mg/kg).

Table 4. 4 Tocol concentration (mg/kg of oil) in the original, column stripped, and stripped by stationary phase (2 h) of camelina, chia and sophia seed oils^{1,2}.

tocols	non-stripped	Column stripped	Stripped by stationary phase (2h)
Camelina Seed Oil			
α -tocopherol	114.73 \pm 2.951 ^a	nd	nd
β -/ γ -tocopherol	677.40 \pm 17.732 ^a	nd	nd
δ -tocopherol	470.41 \pm 48.213 ^a	nd	nd
Total tocols	1262.54 \pm 68.896	nd	nd
Chia Seed Oil			
α -tocopherol	10.94 \pm 1.653 ^a	nd	nd
β -/ γ -tocopherol	282.68 \pm 1.213 ^a	nd	nd
δ -tocopherol	47.44 \pm 1.213 ^a	nd	nd
Total tocols	341.06 \pm 4.079	nd	nd
Sophia Seed Oil			
α -tocopherol	tr	nd	nd
β -/ γ -tocopherol	977.52 \pm 4.372 ^a	nd	nd
δ -tocopherol	173.41 \pm 5.985 ^a	nd	tr
Total tocols	1150.93 \pm 10.357	nd	tr

¹Values are mean of triplicate determination \pm standard deviation. Values with different superscript in each row are significantly different from each other (P<0.05). ²Abbreviation: nd, not detected; and tr, trace (<0.02).

For chia seed oil, the total tocopherol content was 341 mg/kg which is lower than that found in literature (480 mg/kg) by Ixtaina et al (2012), but was within the range reported by Muñoz, Cobos, Diaz and Aguilera (2013) (238-427 mg/kg). The amount of α -tocopherol (10 mg/kg) and δ -tocopherol (47 mg/kg) in this study was higher than the literature values of α -, and δ -tocopherols which were 2 and 15 mg/kg of oil, respectively. For sophia oil, the amount of total tocopherols was more than 1145 mg/kg; however, it was lower than the value of 1585 mg/kg reported by Goffman, Thies and Velasco (1999).

Column-stripped and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oils were devoid of any tocopherol. Based on the above results, the column stripping method and the stripped by stationary phase (2 h) procedure were effective in removing the tocopherols. Therefore, oxidative stability test was conducted for non-stripped, column-stripped and stripped by stationary phase (2 h) for camelina, chia, and sophia seed oils in order to estimate the effects of minor components on their oxidation.

4.2.2. Pigments determination in the original, column stripped, and stripped by stationary phase (2 h) of camelina, chia and sophia seed oils

Pigments, including chlorophylls and carotenoids, were detected spectrophotometrically in the range of 550-710 and 430-460 nm, respectively (Blekas, Tsimidou & Boskou, 1995). The content of carotenoids in non-stripped camelina seed oil was much higher than those present in non-stripped chia, and non-stripped sophia seed oils. However, chlorophylls existed in higher levels in non-stripped sophia seed oil than in camelina and chia seed oils (Figure 4.2), which may influence their oxidative stability under light. It is noteworthy that column stripped as well as

stripped by stationary phase (2 h) of camelina, chia, and sophia seed oils did not contain any pigments.

By comparing the pigments found in non-stripped oils and their counterparts, it is very clear that stripped oils prepared by using the two methods (column stripped and stripped by stationary phase 2 h) had no or very small amounts of ($p < 0.05$) pigment as compared to their non-stripped counterparts (Figure 4.2) as indicated by the absorbance of the associated pigments at different wavelengths. For instance, absorbance at 430 nm for non-stripped camelina seed oil was 0.73, which is higher ($p < 0.05$) than that of its column stripped counterpart (0.017) and that produced by using the stationary phase material for 2 h of contact time (0.014). Similarly, the absorbance at 460 nm for non-stripped sophia seed oil was 0.64, which is much higher ($p < 0.05$) than that of its column stripped and stripped by stationary phase (2 h) (0.01 and 0.02), respectively.

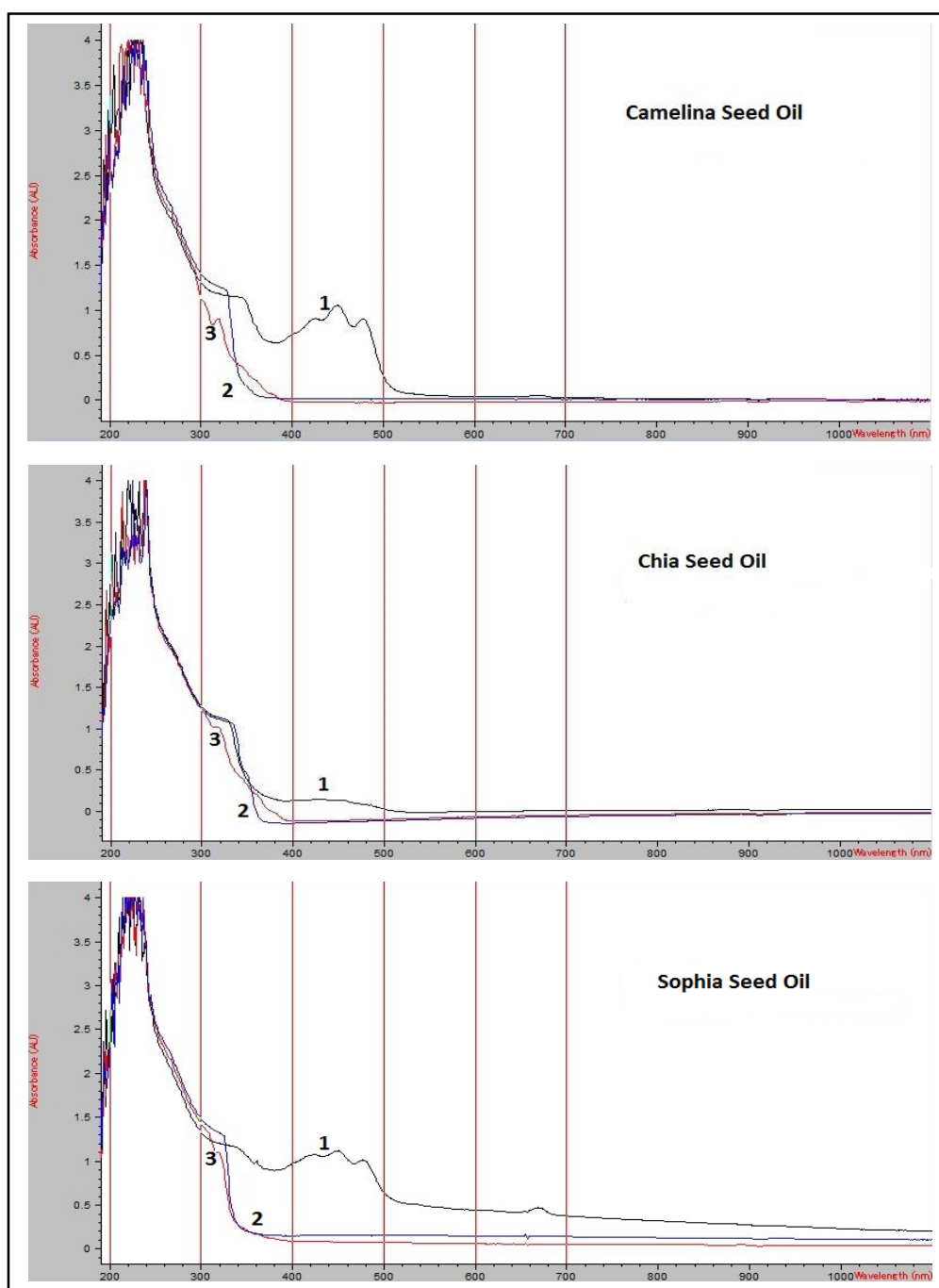


Figure 4.2 Visible spectra of pigments in seed oils (1) original oil, (2) stripped by stationary phase (2 h), and (3) column stripped.

4.3. The oxidative stability of the original, column stripped, and stripped by stationary phase (2 h) of camelina, chia and sophia seed oils

4.3.1. Autoxidation of original, column stripped, and tripped by stationary phase (2 h) of camelina, chia, and sophia seed oils and formation of primary and secondary oxidation products under Schaal oven condition

4.3.1.1. Primary products under Schaal oven condition

The Schaal oven condition is an accelerated oxidation test commonly used to examine the oxidative stability of edible oils and their respective shelf life. During this test (Schaal oven), the oil samples were placed in a forced air oven maintained between 60 and 70°C (Frankel, 1993). It has been shown that one-day storage of oils under Schaal oven condition at 65°C equals to that at room temperature for one month (Abou-Gharbia, Shahidi, Shehata & Youssef, 1996; Pimpa, Kanjanasopa & Boonlam, 2009). In addition, Warner, Frankel and Mounts (1989) reported that flavour scores of edible oils at 60°C for 4 days agreed with those stored at ambient temperature for 4 months.

All oil samples were carefully handled to minimize exposure to light during the course of the experiments. Figure 4.3 shows that all stripped camelina, chia, and sophia seed oils (column stripped, and tripped by stationary phase 2 h) were less stable than their non-stripped counterparts under Schaal oven condition. In addition, all stripped oils reflected similar trends as non-stripped oils. This is probably due to the removal of the minor components which function as antioxidants against oxidation of unsaturated fatty acids. Therefore, minor antioxidative

components present in tested seed oils, such as tocopherols, chlorophylls, and carotenoids played a significant role in stabilizing the non-stripped oils. Since all tested seed oils contain very high amounts of unsaturated fatty acids, free radical chain reactions that may occur could be interrupted by the action of the tocopherols, and thus terminating the propagation reactions (Porter, Caldwell & Mills, 1995). The tocopherols are gradually consumed during the induction period, thus leading to rapid oxidation of stripped oils (Jung & Min, 1990; Baldioli, Servili, Perretti & Montedoro, 1996).

Furthermore, results showed no significant difference between the stripped by column chromatography and stripped by stationary phase (2 h) regarding the oxidative stability during a 7-day storage at 60°C under Schaal oven condition.

Stripped camelina seed oil prepared by the two stripping methods (column stripped and stripped by stationary phase 2 h) showed the least stability towards autoxidation, followed by stripped sophia seed oil and stripped chia seed oil. Interestingly, non-stripped camelina seed oil displayed the best stability during autoxidation, again followed by non-stripped sophia seed oil and non-stripped chia seed oil.

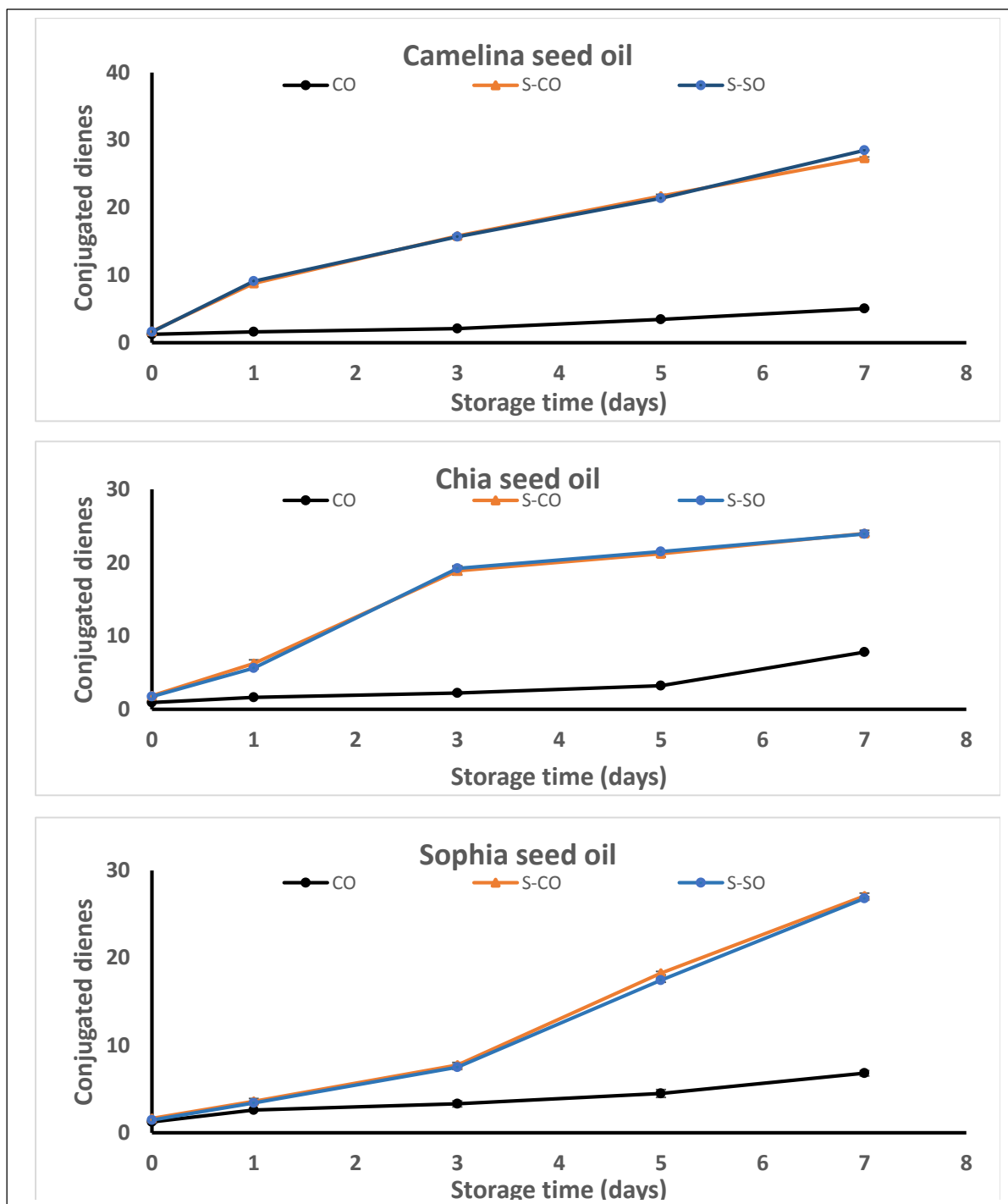


Figure 4.3 Conjugated dienes of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of camelina, chia, and sophia oils seed during a 7-day storage at 60°C under Schaal oven condition ($p > 0.05$).

The results of tocol determination indicated that camelina seed oil contained the highest amount of tocopherol (1262 mg/kg oil, primarily β -/ γ -tocopherol 677 mg/kg oil), which was higher than sophia seed oil (1150 mg/kg of oil) as well as chia seed oil (341 mg/kg of oil). This explains why non-stripped camelina was most stable during autoxidation, while it became the least stable oil after the removal of its tocols. Therefore, tocols and other minor components present exert an impact on the quality of edible oils as well as their corresponding storage quality and shelf life.

4.3.1.2. Secondary oxidation products under Schaal oven condition

During autoxidation, secondary oxidation products of non-stripped, column-stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oils were determined by examining the production of TBARS. This test is based on the determination of colour intensity of the TBA-MDA at 532 nm and expresses the results as μ mol malondialdehyde equivalents per gram (g) of oil.

Figure 4.4 shows the trend for the formation of secondary oxidation products in tested camelina, chia, and sophia seed oils under Schaal oven conditions. All three samples of stripped and non-stripped oils shared a similar trend to conjugated dienes formation demonstrating that stripped oils were less stable than their non-stripped counterparts. The formation of secondary oxidation products increased rapidly after the first day, but upon further storage, changes occurred in the TBARS values; however, this was not tested due to advanced oxidation which caused solidification of the oils used due to their polymerization after 10 days. This made it impossible to test them for both CD and TBARS evaluation.

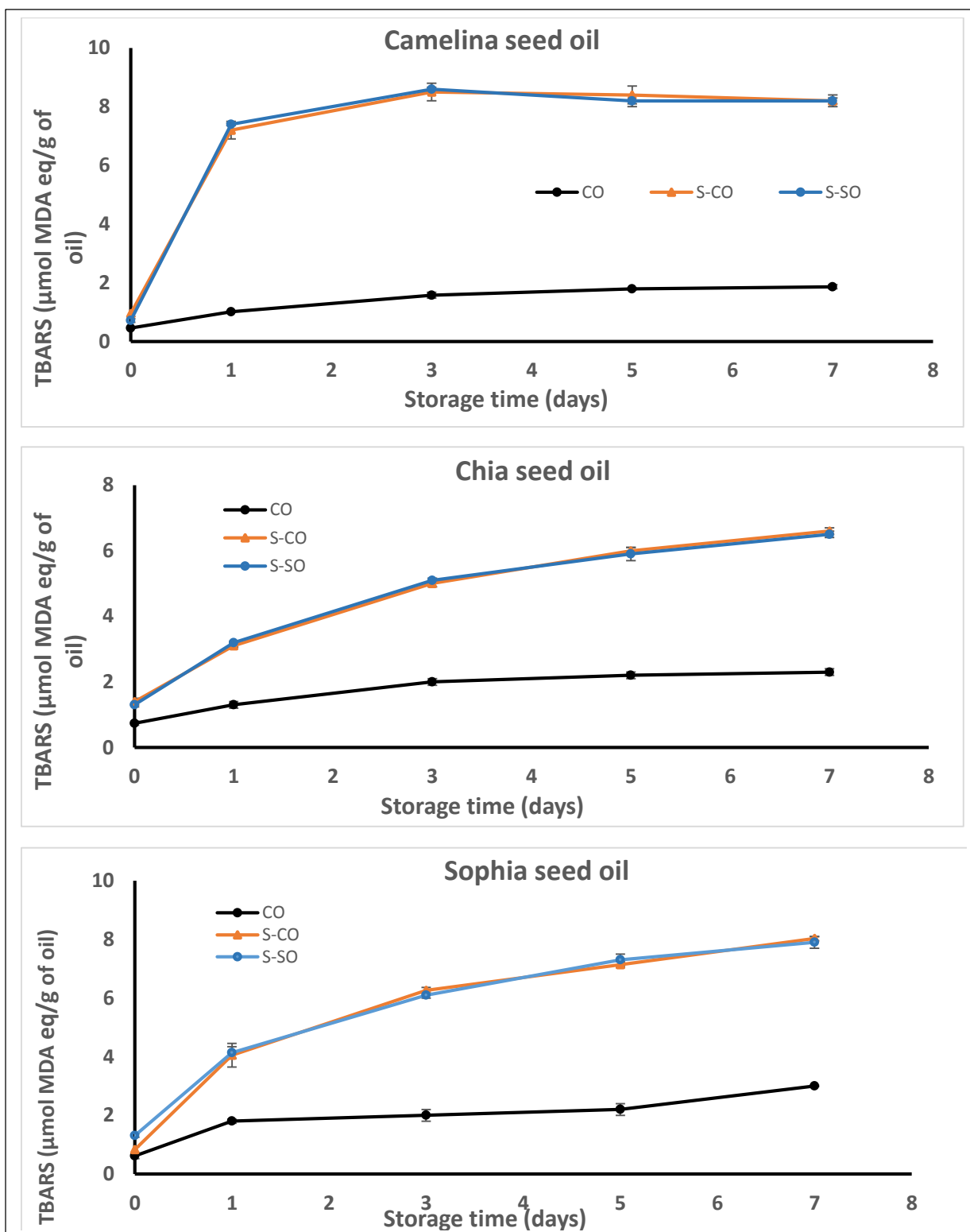


Figure 4.4 Thiobarbituric acid reactive substances (MDA equivalents) of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of camelina, chia, and sophia oil seed during a 7-day storage at 60°C under Schaal oven condition ($p > 0.05$).

The examination of TBARS conducted during autoxidation showed that chia and sophia oils prepared by the two stripping methods (column stripped, and stripped by stationary phase, silicic acid/charcoal; 2 h) were less stable than stripped camelina seed oil. This demonstrates the importance of minor components on the stability and oxidation of chia and sophia seed oils. Non-stripped chia oil showed the best oxidative stability among all tested oils, both stripped and non-stripped; this might be due to the depletion of tocopherols. Under Schaal oven condition, large amounts of tocopherols in camelina and sophia, compared to those in chia seed oil, were gradually consumed during the induction period in both cases, thus leading to a rapid oxidation. Hence, the stabilities of the tested camelina, chia, and sophia seed oils depended largely on the remaining amount of tocopherols in the samples.

4.3.2. Assessment of oxidative stability of original, column stripped, and stripped by stationary phase (2 h) of camelina, chia and sophia seed oils under fluorescent light

4.3.2.1. Primary oxidation products under fluorescent light

Figure 4.5 presents the trend for the formation of the primary oxidation products during photooxidation by measuring the conjugated dienes (CD) in non-stripped, column stripped, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oils. As seen in Figure 4.5, all stripped seed oils were significantly more stable than their non-stripped counterparts. Meanwhile, all three samples of non-stripped and stripped oils followed a similar trend in the formation of conjugated dienes (CD), which showed a rapid increase for conjugated dienes of non-stripped oils compared with their stripped counterparts. It is worth mentioning that there

was no significant difference between the column stripped oils, and those stripped by the stationary phase material (2 h) (Figure 4.5). Therefore, non-stripped oils were more susceptible to oxidation than their stripped counterpart samples, which is a reverse of that for the autoxidation results. Other work done in our lab with different samples (Abuzaytoun & Shahidi, 2006; Li, Wang & Shahidi, 2016) also noted that the presence of pigments, mainly chlorophylls and carotenoids, in non-stripped oils may be a possible reason for this observation. Chlorophylls are photosensitizers that lead the oils to photooxidation even in the presence of tocopherols. Non-stripped tested camelina seed oil showed the least stability against photooxidation which indicated that chlorophylls are responsible for photooxidation of the oils since camelina seed oil contained the highest amount of chlorophylls; even though it also contained the highest amount of tocopherols. Thus, the presence of chlorophylls still prevails over the antioxidant effect of the tocopherols.

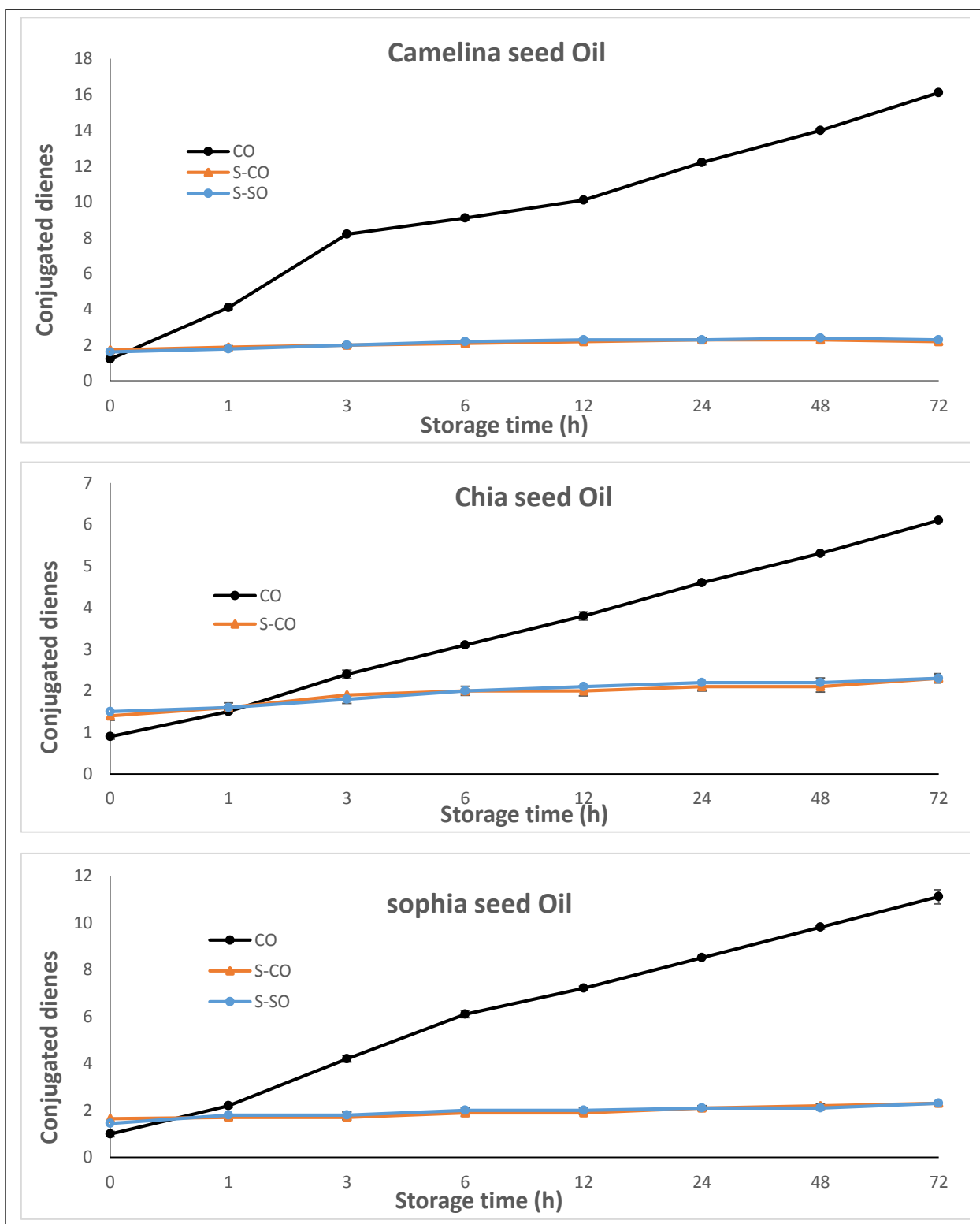


Figure 4.5 Conjugated dienes of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2h) (S-SO) of camelina, chia, and sophia seed oils during 72 h storage period under fluorescent lighting at 27 °C ($P < 0.05$).

Warner and Frankel (1987), as well as Hall and Cuppett (1993) reported that both chlorophylls and carotenoids act as photosensitizers which lead to the formation of hydroperoxides that subsequently decompose to volatiles causing rancidity or oxidative deterioration of edible oils. The role of photosensitizers in light-induced oxidation has also been observed in non-stripped borage and evening primrose oils, non-stripped hemp and flax seed oils, and berry seed oils (Khan & Shahidi, 2002; Abuzaytoun & Shahidi; 2006; Li, Wang & Shahidi, 2016). However, the pigments detected in tested seed oils include chlorophylls as well as carotenoids, so the carotenoids can function as singlet oxygen quenchers during oxidation (Rahmani & Csallany, 1991). It is noteworthy that traces of the carotenoids may be retained in stripped seed oils that could be involved in oxidation, as the instability of carotenoids especially β -carotene under light or heat needs to be considered (Subagio & Morita, 2001).

4.3.2.2. Secondary oxidation products under fluorescent light

The secondary oxidation products as reflected in TBARS values of non-stripped, stripped by column, and stripped by stationary phase (2 h) of camelina, chia, and sophia seed oils under fluorescent light are illustrated in Figure 4. Which indicates that non-stripped chia seed oil had better stability than other oils. According to the data presented, non-stripped as well as stripped chia seed oil had the best stability compared to the other samples during the first 24-hour period.

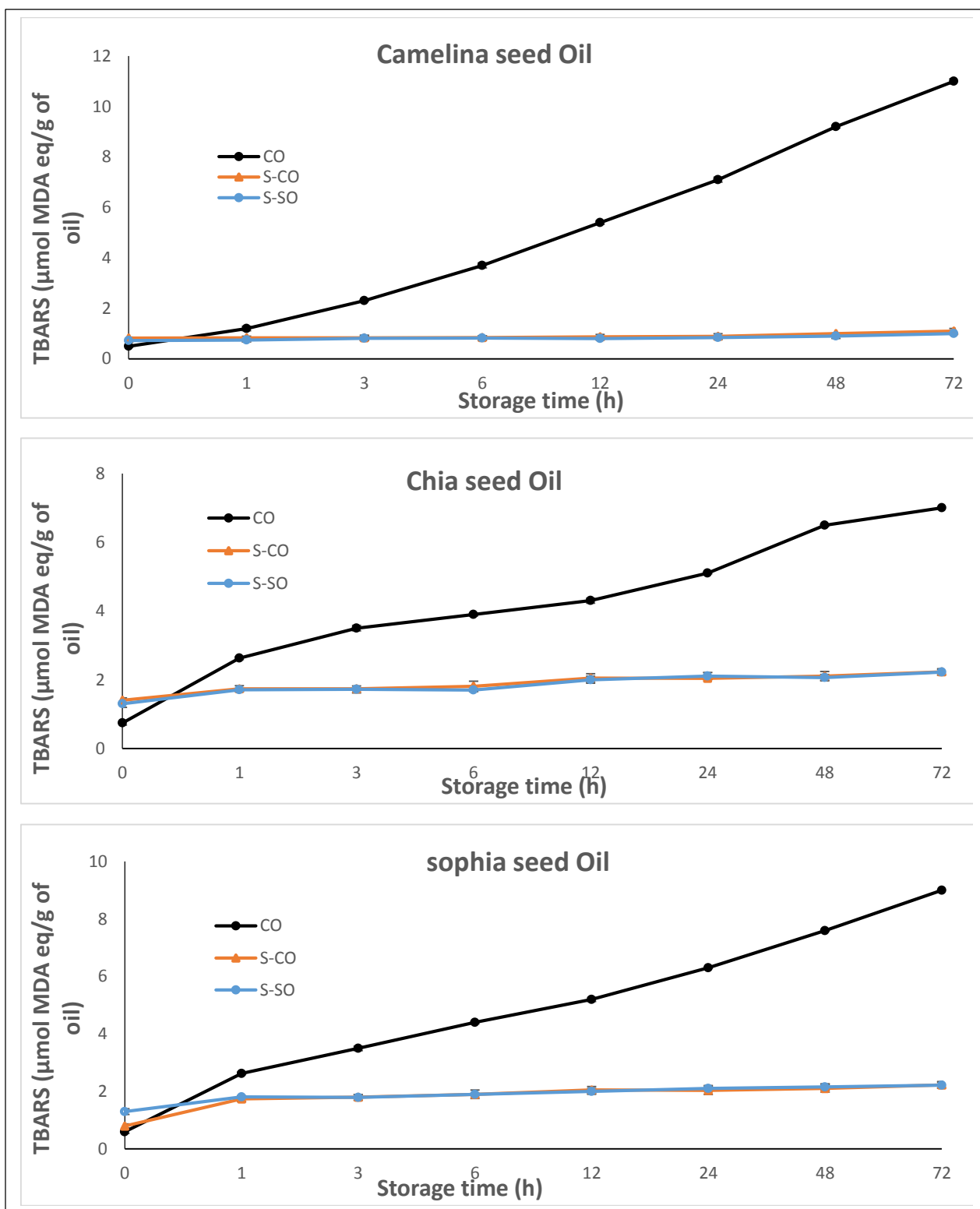


Figure 4.6 Thiobarbituric acid reactive substances (MDA equivalents) of crude oil (CO stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of camelina, chia, and sophia seed oil during a 72-hour storage under fluorescent light at 27 °C ($P > 0.05$).

CHAPTER 5

CHAPTER 5

5.1. The optimization of new stripping method using stationary phase (silicic acid/charcoal) material

Several chromatographic techniques have been used to strip different oils from their minor components. Column chromatographic methods that use packed columns with silicic acid and charcoal in different proportions were quite effective in removing minor components from all tested oils (Mistry & Min, 1988; Khan & Shahidi, 2000; Abuzaytoun & Shahidi, 2006; Lampi et al., 1992; Tian, Decker & Goddard, 2013; Li, Wang & Shahidi, 2016). However, the process is laborious and takes a long time to achieve its goal. From this point, a novel and simplified stripping method using stationary phase components was employed. In short, 60 g of oil diluted with 60 ml of hexane was stirred with the same packing material used in the column (90 g of activated silicic acid and 45 g of charcoal); this mixing operation was carried out in a fume hood at 27.5⁰C, followed by suction filtration. This method was performed for different time periods 1 hour, 2 hours, and also for a second time 1 hour each time.

In this chapter, first the novel and simplified stripping method using stationary phase material for different time (for 1 hour, 2 hours, and for the second time 1 hour each time) was compared with column stripping of camelina, chia, and sophia seed oils for determining the minor components such as antioxidants (tocopherols) and pigments (chlorophylls and carotenoids). Secondly, the efficiency of the two stripping methods was examined for removing the minor components from other oil samples including fruit oil (olive), vegetable oil (corn), and

algal oil (DHASCO). In addition, it examined their effects on the oxidative stability of these latter oils.

5.2. Assessment of minor components

5.2.1. Assessment of tocols in the original, column stripped and stripped by stationary phase 1 h, 2 h, and for second time (1 hour each time) of camelina, chia and sophia seed oils

The concentrations of tocols in camelina, chia, and sophia seed oils and their striped counterparts using column stripping and stripping by stationary phase for 1 h, 2 h, and for a second time 1 hour each time are shown in Table 5.1. The total tocopherol contents of non-stripped camelina, chia, and sophia seed oils differed and were 1262.54, 341.06, and 1150.93 mg/kg of oil, respectively (Also see Chapter 4).

Based on the results presented in Table 5.1, the column-stripped and stripped by stationary phase (2h) camelina, chia, and sophia seed oils were devoid of any tocopherols. However, oils stripped by stationary phase for 1h, still retained some tocopherol; 20.79 mg/kg in camelina which was higher than those in sophia (3.63 mg/kg) and chia seed oils (0.01 mg/kg), thus this condition proved inadequate for total removal of tocopherols.

For oils stripped by stationary phase (twice) also retained some tocopherols in camelina and sophia, but no tocopherols remained in chia seed oil. After comparing all results, column-stripped and stripped by stationary phase for 2h methods were both found to completely remove the tocopherols.

Table 5. 1 Tocol concentration (mg/kg of oil) in the original, column stripped, and stripped by stationary phase (1 h, 2 h, and twice) of camelina, chia, and sophia seed oils^{1,2}.

tocols	non-stripped	Column stripped	Stripped by stationary phase (1h)	Stripped by stationary phase (2h)	Stripped by stationary phase (twice)
Camelina Seed Oil					
α -tocopherol	114.73 \pm 2.951 ^a	nd	8.26 \pm 0.581 ^b	nd	2.10 \pm 0.00 ^c
β -/ γ -tocopherol	677.40 \pm 17.732 ^a	nd	12.53 \pm 0.008 ^b	nd	4.21 \pm 0.110 ^c
δ -tocopherol	470.41 \pm 48.213 ^a	nd	nd	nd	nd
Total tocols	1262.54 \pm 68.896 ^a	nd	20.79 \pm 0.589 ^b	nd	6.31 \pm 0.110 ^c
Chia Seed Oil					
α -tocopherol	10.94 \pm 1.653 ^a	nd	nd	nd	nd
β -/ γ -tocopherol	282.68 \pm 1.213 ^a	nd	nd	nd	nd
δ -tocopherol	47.44 \pm 1.213 ^a	nd	0.01 \pm 0.012 ^b	nd	nd
Total tocols	341.06 \pm 4.079 ^a	nd	0.01 \pm 0.012 ^b	nd	nd
Sophia Seed Oil					
α -tocopherol	tr	nd	nd	nd	nd
β -/ γ -tocopherol	977.52 \pm 4.372 ^a	nd	2.14 \pm 0.011 ^b	nd	1.50 \pm 0.012 ^c
δ -tocopherol	173.41 \pm 5.985 ^a	nd	1.47 \pm 0.133 ^c	tr	2.73 \pm 0.012 ^b
Total tocols	1150.93 \pm 10.357 ^a	nd	3.63 \pm 0.144 ^c	tr	4.23 \pm 0.023 ^b

¹Values are mean of triplicate determination \pm standard deviation. Values with different superscript in each row are significantly different from each other (P<0.05). ²Abbreviation: nd, not detected; and tr, trace (<0.02).

5.2.2. Pigments determination in the original, column stripped and stripped by stationary phase 1 h, 2 h, and for second time (1 hour each time) of camelina, chia and sophia seed oils

In general, the content of carotenoids in non-stripped camelina seed oil was much higher than those present in both non-stripped chia and sophia seed oils. However, chlorophylls were present at higher levels in non-stripped sophia seed oil than camelina and chia seed oils (Figure 5.1). It is noteworthy that column stripped, stripped by stationary phase 1 h, 2 h, and for the second time of camelina, chia, and sophia seed oils did not show the presence of any pigments.

By comparing the pigments found in non-stripped oils and their counterparts, it is clear that stripped oils by all methods used in this study (column stripped, stripped by stationary phase 1 h, 2 h, and for the second time) had non or very small amounts of pigment compared their non-stripped counterparts ($p < 0.05$; Figure 5.1) as indicated by the absorbance of pigments at different wavelengths. For example, the absorbance at 430 nm for non-stripped sophia seed oil was 0.75, which is significantly higher ($p < 0.05$) than the oil stripped by column (0.013) and by stationary phase 1 h (0.20), 2 h (0.15), and for the second time (0.016). Similarly, the absorbance at 460 nm for non-stripped chia seed oil was 0.23, which is much higher ($p < 0.05$) than that of its column stripped and stripped by stationary phase for 1 h, 2 h, and for a second time (0.03, 0.015, and 0.016, respectively).

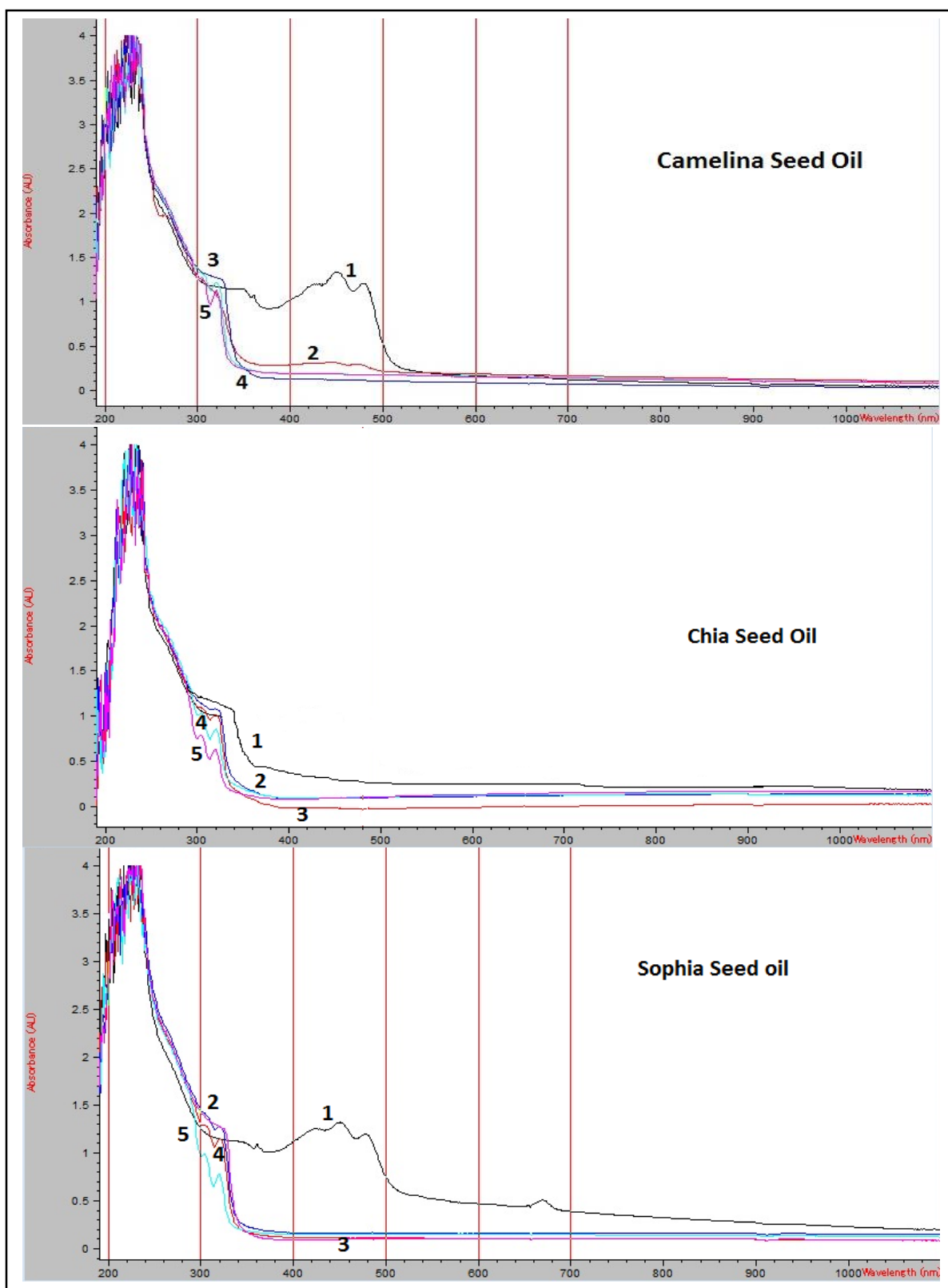


Figure 5.1 Visible spectra of pigments in seed oils (1) original oil, (2) stripped by stationary phase 1 h, (3) stripped by stationary phase 2h, (4) stripped by stationary phase (twice), and (5) column stripped.

5.3. Assessment of minor components in corn oil, olive oil and algal oil (DHASCO)

To examine the efficiency of the stripping methods employed in this work to remove the minor components of oils, we selected several oil samples to expand its application using fruit oil (olive), a vegetable oil (corn), and an algal oil (DHASCO). In addition, we determined their effects on the oxidative stability of both non-stripped and stripped oil samples mentioned above.

5.3.1. Assessment of tocopherols in the original, column stripped, and stripped by stationary phase 1 h, 2 h, and for second time (1 hour each time) of corn oil, olive oil, and algal oil (DHASCO)

The concentration of tocopherols in corn oil, olive oil, and algal oil (DHASCO) and their stripped counterparts using column stripping, stripped by stationary phase 1 h, 2 h, and the second time are shown in Table 5.2. The non-stripped corn oil, olive oil, and algal oil (DHASCO) differed in their α -, β -/ γ -, δ -, and total content of tocopherols and were 538.89, 208.28, and 1211.98 mg/kg of oil, respectively.

The main tocopherols in DHASCO, as determined by HPLC, were α -tocopherol (759.28 mg/kg), δ -tocopherol (250.63 mg/kg) and β -/ γ -tocopherol (202.07 mg/kg); however, corn oil contained 64.47 mg/kg of α -tocopherol, 445.99 mg/kg of β -/ γ -tocopherol, and 28.43 mg/kg of δ -tocopherol. Olive oil contained 179.98 mg/kg of α -tocopherol, 28.30 mg/kg of β -/ γ -tocopherol, and no δ -tocopherol was detected as shown in Table 5.2. Thus, DHASCO had higher amounts ($P < 0.05$) of total tocopherols than corn and olive oils, which might contribute to greater enhancement of its oxidative stability.

Table 5. 2 Tocol concentration (mg/kg of oil) in the original, column stripped, and stripped by stationary phase (1 h, 2 h, and twice) of corn oil, olive oil, and algal oil (DHASCO)^{1,2}.

tocols	non-stripped	Column stripped	Stripped by stationary phase (1h)	Stripped by stationary phase (2h)	Stripped by stationary phase (twice)
Corn Oil					
α -tocopherol	64.47 \pm 3.261 ^a	nd	Nd	nd	nd
β -/ γ -tocopherol	445.99 \pm 4.652 ^a	nd	Nd	nd	nd
δ -tocopherol	28.43 \pm 4.813 ^a	nd	0.61 \pm 0.011 ^b	nd	nd
Total tocols	538.89 \pm 12.786 ^a	nd	0.61 \pm 0.011 ^b	nd	nd
Olive Oil					
α -tocopherol	179.98 \pm 7.094 ^a	nd	Nd	nd	0.89 \pm 0.012 ^b
β -/ γ -tocopherol	28.30 \pm 7.504 ^a	nd	Nd	nd	nd
δ -tocopherol	nd ^a	nd	Nd	nd	nd
Total tocols	208.28 \pm 14.598 ^a	nd	Nd	nd	0.89 \pm 0.012 ^b
Algal oil (DHASCO)					
α -tocopherol	759.28 \pm 3.101	nd	Nd	nd	nd
β -/ γ -tocopherol	202.07 \pm 3.891 ^a	nd	Nd	nd	6.12 \pm 0.022 ^b
δ -tocopherol	250.63 \pm 1.361 ^a	nd	Nd	tr	2.17 \pm 0.021 ^b
Total tocols	1211.98 \pm 8.353 ^a	nd	Nd	tr	8.29 \pm 0.023 ^b

¹Values are mean of triplicate determination \pm standard deviation. Values with different superscript in each row are significantly different from each other (P<0.05). ²Abbreviation: nd, not detected; and tr, trace (<0.02).

The results obtained confirmed that stripped corn oil, olive oil, and DHASCO oil prepared by column stripping and stripped by stationary phase for 2 h were devoid of any tocopherol, therefore the methods employed were effective in removing tocopherols. However, the samples that were stripped using other methods still retained a small amount of tocopherols.

5.3.2. Pigments determination in the original, column stripped and stripped by stationary phase 1 h, 2 h and for the second time (1 hour each time) of corn oil, olive oil and algal oil (DHASCO)

Pigments like carotenoids, with absorbances between 430 and 460 nm (Blekas et al., 1995), were present in high amounts in olive oil and algal oil (DHASCO) (Figure 5.2). Meanwhile, chlorophylls, which absorb light between 550 and 710 nm (Blekas et al., 1995), were present in a higher quantity in olive oil than corn oil. On the other hand, no chlorophylls were found in non-stripped DHASCO. By comparing pigments found in corn oil, olive oil, and algal oil (DHASCO) with their respective counterparts, it is clear that non-stripped oils had more ($p < 0.05$) pigments than their stripped counterparts (Figure 5.2) as indicated by the absorbance of pigments at different wavelengths. For example, the absorbance at 430 nm for DHASCO was 1.16 which was higher $p < 0.05$ than that of column stripped, stripped by stationary phase 1 h, 2 h, and for the second time (0.38, 0.52, 0.42, and 0.04), respectively. Similarly, the absorbance at 430 nm for olive oil was 0.82 which was also higher ($p < 0.05$) than that of column stripped, stripped by stationary phase 1 h, 2 h, and for the second time (0.05, 0.09, 0.04, and 0.16), respectively. Meanwhile, stripping of oils by column and by the stationary phase 2 h were more effective compared to other stripping methods.

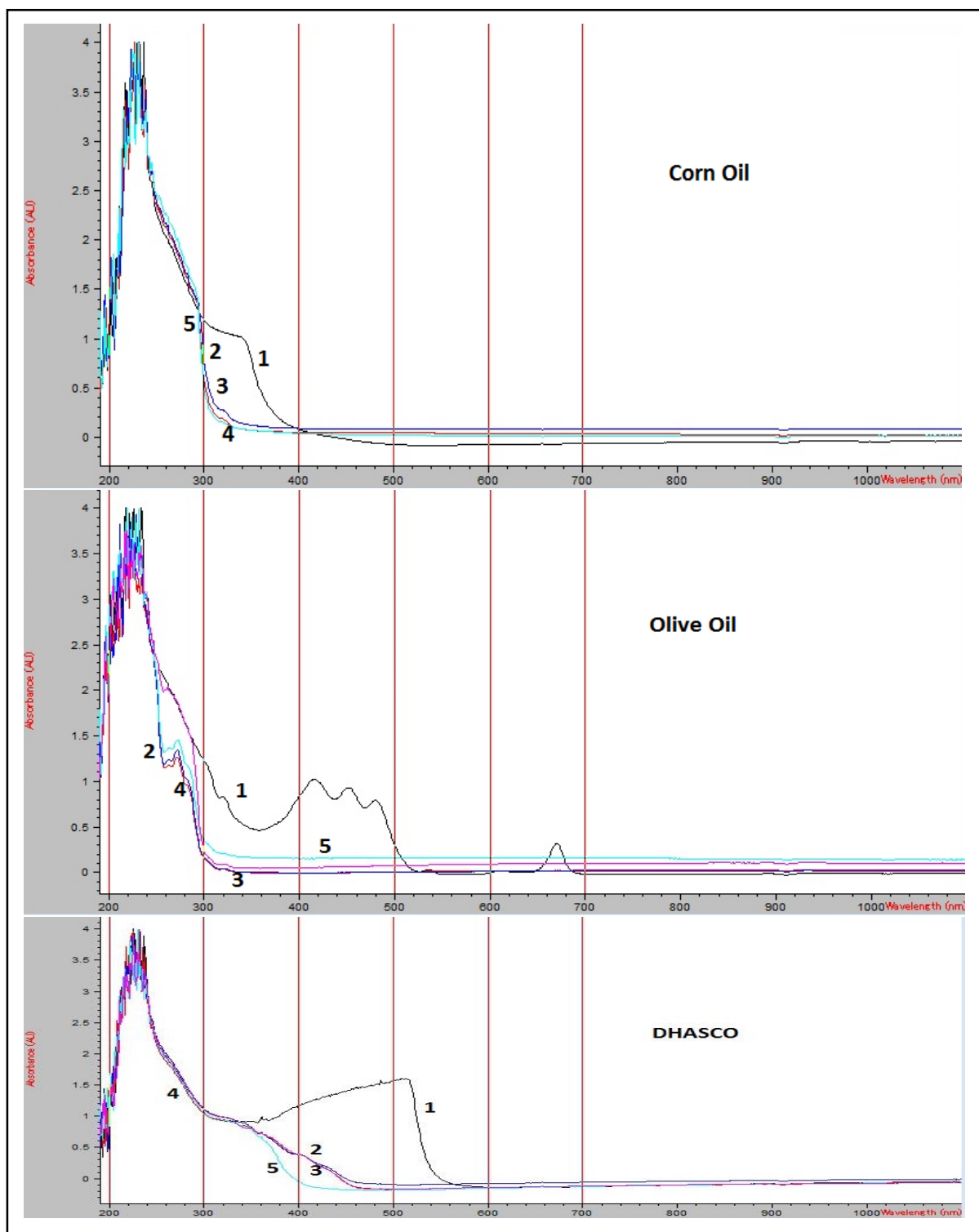


Figure 5.2 Visible spectra of pigments in seed oils (1) original oil, (2) stripped by stationary phase 1 h, (3) stripped by stationary phase 2 h, (4) stripped by stationary phase (twice), and (5) column stripped.

5.4. The oxidative stability of the original, column stripped, and stripped by stationary phase (2 h) of corn oil, olive oil and algal oil (DHASCO)

5.4.1. Autoxidation of original, column stripped, and tripped by stationary phase (2 h) of corn oil, olive oil and algal oil (DHASCO) and formation of primary and secondary oxidation products under Schaal oven condition

5.4.1.1. Primary products under Schaal oven condition

As mentioned in Chapter 4, the Schaal oven method is commonly used to examine the oxidative stability of edible oils and also to predict their shelf life. Figure 5.3 shows that all stripped corn oil, olive oil, and algal oil (DHASCO), both column stripped, and stripped by the stationary phase 2 h, were less stable than their non-stripped counterparts under the Schaal oven conditions; also all stripped oils followed similar trends as non-stripped oils. This is perhaps due to the removal of minor components that work as antioxidants against oxidation of unsaturated fatty acids. Thus, minor antioxidative components present in tested seed oils, such as tocopherols, chlorophylls, and carotenoids played a significant role in stabilizing the non-stripped oils.

Moreover, it is important to note that no significant difference existed between the samples stripped by column chromatography and stripped by the stationary phase materials (2 h) regarding their oxidative stability during a 7-day storage at 60 °C under Schaal oven condition.

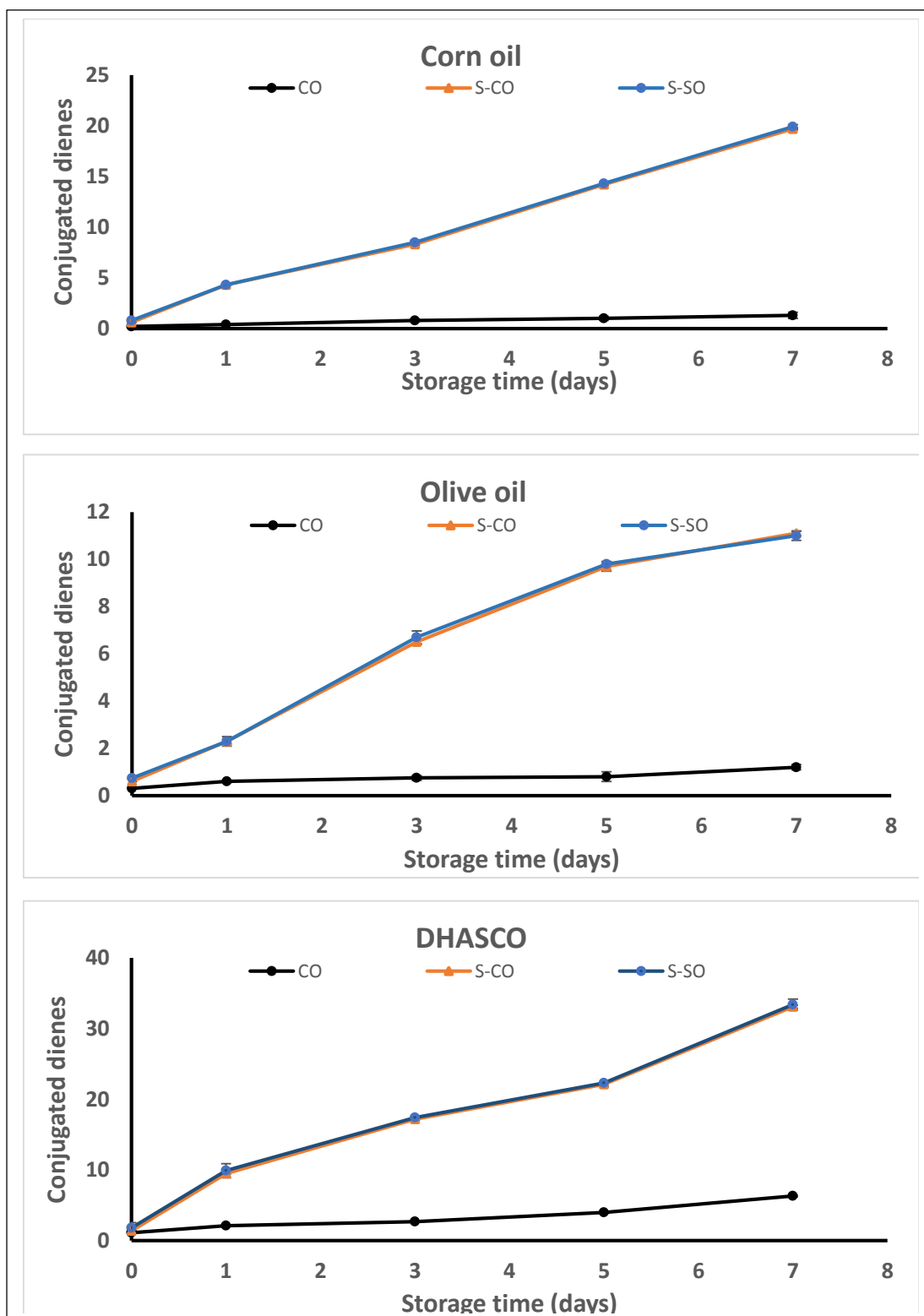


Figure 5.3 Conjugated dienes of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of corn oil, olive oil, and algal oil (DHASCO) during a 7-day storage at 60°C under Schaal oven conditions ($p > 0.05$).

The results of tocol determination indicated that DHASCO contained the highest amount of tocopherol (1211.98 mg/kg of oil, primarily α -tocopherol 759.28), which was higher than corn oil (538.89 mg/kg of oil) as well as olive oil (208.28 mg/kg of oil); this may explain why non-stripped DHASCO was most stable during autoxidation, while it became the least stable after the removal of its tocols. Thus, tocols and other minor components present exert an impact on the quality of edible oils as well as their shelf lives.

5.4.1.2. Secondary oxidation products under Schaal oven condition

During autoxidation, secondary oxidation products of non-stripped, column-stripped, and stripped by stationary phase (2 h) of corn oil, olive oil, and algal oil (DHASCO) were determined by examining TBARS. Figure 5.4 shows the trend for the formation of secondary oxidation products in the three tested oils under Schaal oven condition. All samples of stripped and non-stripped oils followed a similar trend for the formation of conjugated dienes, but stripped oils were less stable than their non-stripped counterparts. The formation of secondary oxidation products increased rapidly after the first day of storage.

The examination of TBARS during autoxidation showed that corn and olive oils stripped by the two methods of column stripping, and stripped by the stationary phase for 2 h were less stable than stripped DHASCO. This indicates the importance of minor components on the stability and oxidation of corn oil and olive oil. Non-stripped olive oil showed the best oxidative stability among all tested oils both stripped and non-stripped; this can be due to the depletion of tocols. Under autoxidation, large amounts of tocols in DHASCO and corn oil, which have more tocols than olive oil, were gradually consumed during the induction period in both cases, leading to a rapid oxidation. Thus, stabilities of the tested oils depend largely on the remaining amount of tocol.

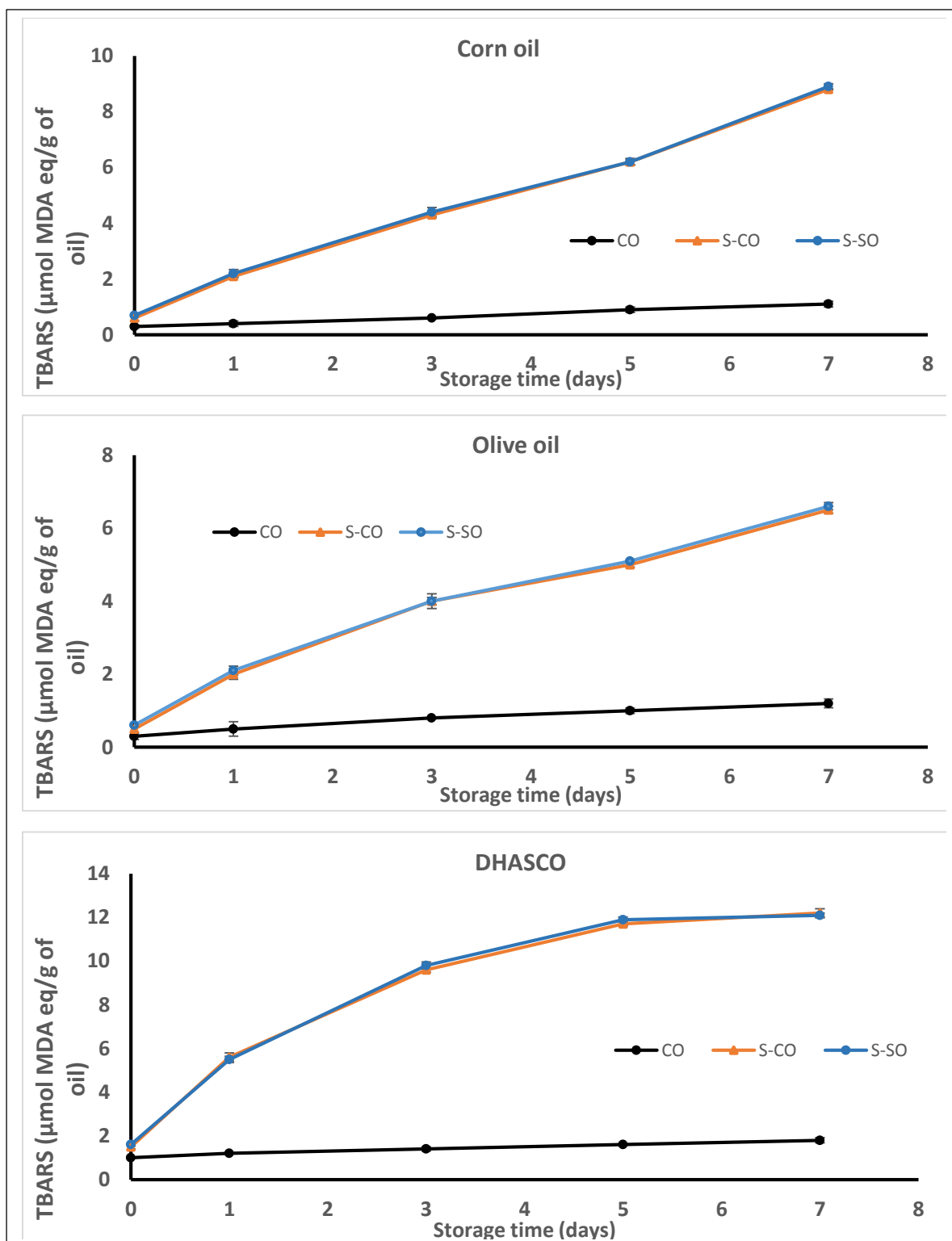


Figure 5.4 Thiobarbituric acid reactive substances (MDA equivalents) of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of corn oil, olive oil, and algal oil (DHASCO) during a 7-day storage at 60 °C under Schaal oven conditions

5.4.2. Assessment of oxidative stability of original, column stripped and stripped by stationary phase (2 h) of corn oil, olive oil and algal oil (DHASCO) under fluorescent light

5.4.2.1. Primary oxidation product

Figure 5.5 presents the trend for the formation of the primary oxidation products during photooxidation by measuring the conjugated dienes (CD) in non-stripped, column stripped, and stripped by stationary phase (2 h) of corn oil, olive oil, and algal oil (DHASCO). All stripped oils were significantly more stable ($p < 0.05$) than their non-stripped counterparts. All samples of non-stripped and stripped corn oil, olive oil, and algal oil (DHASCO) followed a similar trend in the formation of conjugated dienes (CD), which showed a rapid and continuous increase in conjugated dienes of non-stripped oils compared with their stripped counterparts. It is worth mentioning that no significant difference existed between the column stripped oils and those stripped by the stationary phase (2 h) as shown in Figures 5.5. Therefore, non-stripped oils were more susceptible to oxidation than their stripped counterpart samples, which is the reverse of that for the autooxidation results.

Chlorophylls are photosensitizers that lead to photooxidation of oils even in the presence of tocopherols. Non-stripped tested oil showed the least stability against photooxidation indicating that chlorophylls are responsible for the photooxidation of oils since DHASCO contained the highest amount of chlorophylls; even though DHASCO seed oil had the highest amount of tocopherols, but the presence of chlorophylls still prevails over the antioxidant effect of tocopherols.

5.4.2.2. Secondary oxidation products

The secondary oxidation products as reflected in TBARS values of non-stripped, stripped by column, and stripped by stationary phase (2 h) of corn oil, olive oil, and algal oil (DHASCO) under fluorescent light are illustrated in Figure 5.6. The figure indicate that non-stripped corn oil had better stability than other oils. According to the data presented, non-stripped as well as stripped corn oil had the best stability compared to other samples during the first 24-hour of storage period.

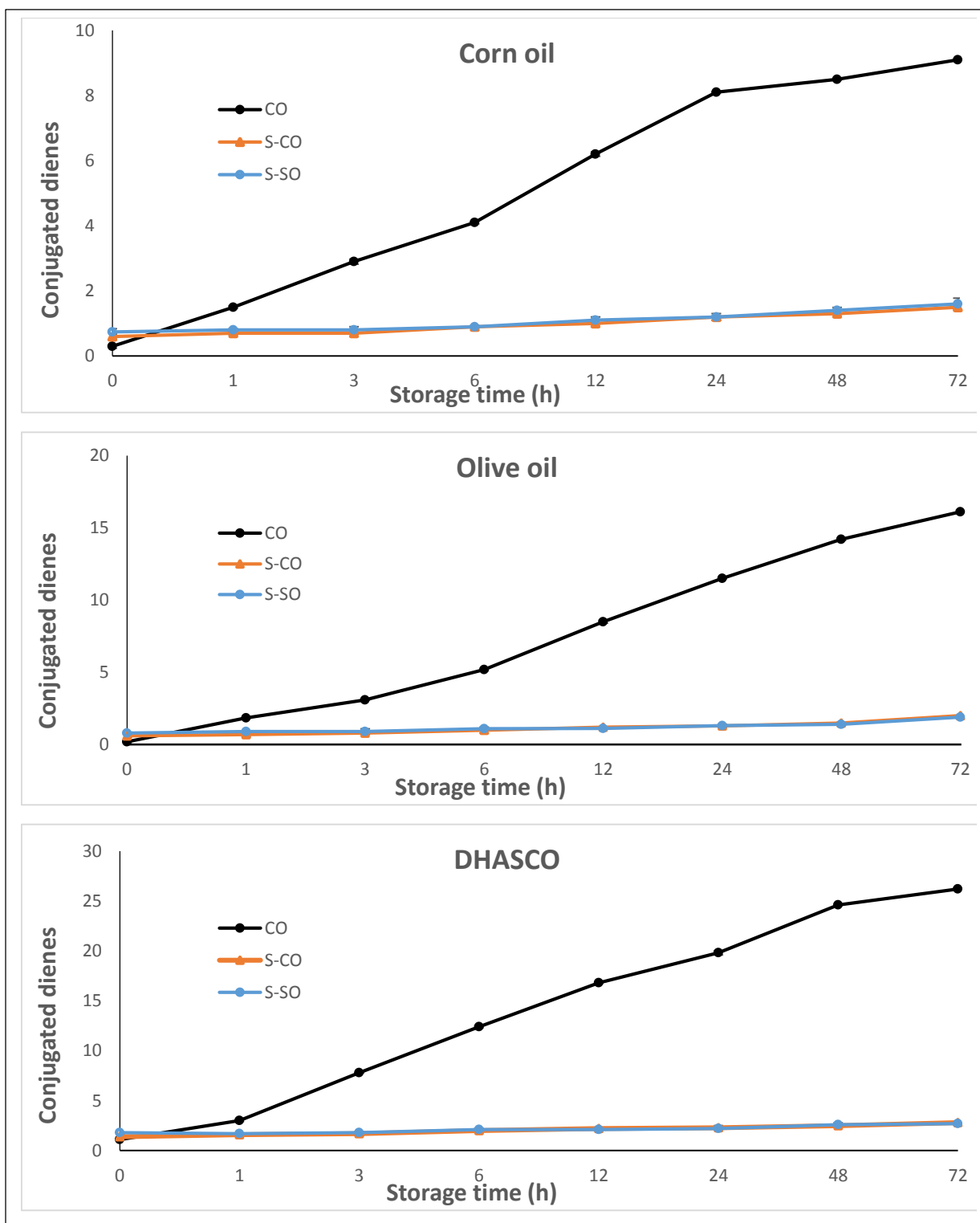


Figure 5.5 Conjugated dienes of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of corn oil, olive oil, and algal oil (DHASCO) during 72 h storage period under fluorescent lighting at 27 °C ($P < 0.05$).

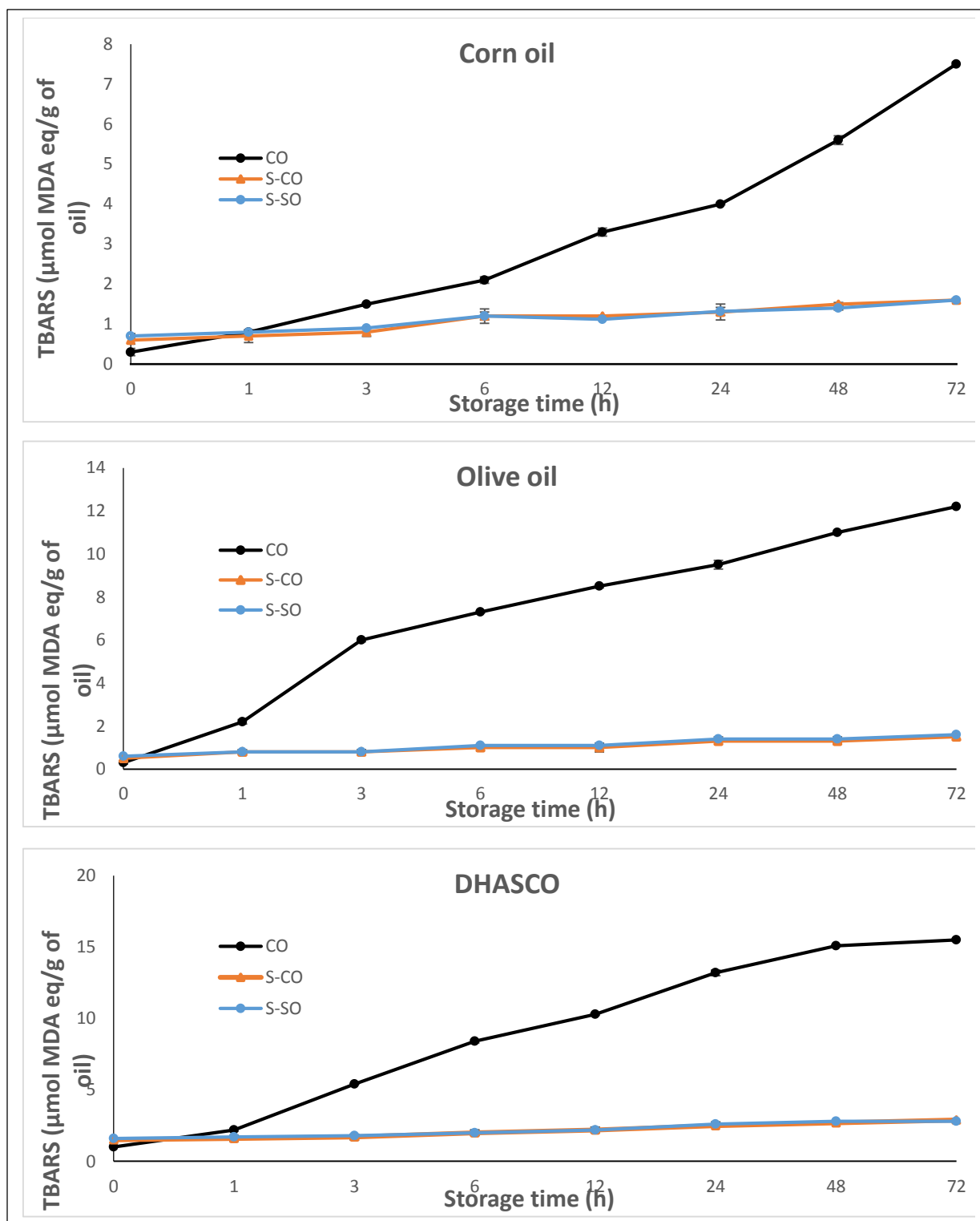


Figure 5.6 Thiobarbituric acid reactive substances (MDA equivalents) of crude oil (CO), stripped by column (S-CO), and stripped by stationary phase (2 h) (S-SO) of corn oil, olive oil, and algal oil (DHASCO) during a 72-hour storage under fluorescent light at 27 °C ($P > 0.05$).

CHAPTER 6

SUMMARY AND RECOMMENDATIONS

The quality of edible oils depends on their chemical characteristics, such as fatty acid composition, triacylglycerol (TAG) profile, and the endogenous antioxidants, such as tocopherols and pigments present. Meanwhile, storage condition, such as light and heat, can also impact the stability of oils. In this connection, camelina, chia and sophia seed oils contained a very simple fatty acid and TAG profiles. Oleic, linoleic and α -linolenic acids were the most prevalent fatty acids in all three seed oils examined. L_nL_nL_n, L_nL_nL_n, L_nL_nP, LLL_n, and L_nOL_n were the dominant TAG species present in the tested oils. Camelina, chia, and sophia seed oils may be used as important dietary sources of essential fatty acids.

Beta-/Gamma-tocopherols were the predominant tocopherols detected in all seed oils. Camelina had the highest content of total tocopherols as well as β -/ γ -tocopherol. Sophia seed oil contained the highest amount of chlorophylls. In order to evaluate the effects of these minor components on oil stability, a comparison of their oxidative stability was made before and after the removal of minor components. This study confirmed that tocopherols had a dominant effect in stabilization of the oils during autoxidative processes but were overwhelmed by the effect of chlorophylls under photooxidative storage conditions. This work also compared two techniques for the stripping of minor components from the oils; these were the commonly used column chromatography and a novel and simplified method that stripped the oil by stationary phase (silicic acid/charcoal; 2 h); both methods successfully removed the minor components, thus the facile stripping method in this work makes an important contribution for removing the minor

components from oils in order to demonstrate their role in stability and stabilization of edible oils

The oxidative stability under accelerated oxidation tests was dictated by the presence and contents of polyunsaturated fatty acids (PUFAs) and minor components including tocopherols and pigments. During autooxidation (under Schaal oven conditions), non-stripped seed oils displayed a better stability than their stripped counterparts. However, under the photooxidation storage conditions, a reverse trend was observed in that stripped seed oils exhibited a better stability than their non-stripped counterparts. Therefore, tocopherols worked as antioxidants during autooxidation, whereas pigments worked dominantly as photosensitizers during photooxidation. Based on the results presented, these oils, similar to extra virgin olive oil, must be protected from light by using tins or dark bottles in order to retain their health promoting minor components and prevent off-flavour development.

This study has provided detailed information about the chemical characteristics of camelina, chia, and sophia seed oils which can be used to explore potential use of these oils in both food and non-food products. In addition, their TAG profiles were revealed for the first time, which can be used as a reference in future studies as well as predicting their products following pancreatic digestion. The chemical compositions of camelina, chia, and sophia seed oils demonstrate their potential health benefits and application areas. These oilseeds provide new trends for product development of the oil-based nutraceuticals and/or supplements which could promote human health. From a nutritional point of view, the high unsaturated fatty acid content in these seed oils makes them potentially suitable alternatives to other highly unsaturated oils in food and feed applications. Meanwhile, camelina, chia, and sophia seed oils may exert important effects such as cholesterol-lowering activity and prevention of cardiovascular disorders.

Further research is needed in detailed determination of the bioactive components in camelina, chia, and sophia seeds and their oils to develop novel application for their use for optimum human nutrition and health. Moreover, the leftover meals, including their outer layer (skin) after oil extraction, might be evaluated for the presence of bioactive components and their potential use in food applications. The presence and contents of glucosinolates and their breakdown products in camelina and sophia seed meals might also help to better design for the use of the left-over meals for use in human food and animal feed. A better understanding of the proteins present in these oilseeds might also provide important information for their effective, value-added utilization.

Finally, this study demonstrated that camelina and sophia seed oils contained significant levels of natural antioxidants. Thus, they may serve as relatively stable oils for specialty food applications while their meals might be used as potential protein extenders with high antioxidant potential in formulated meat and seafood products with improved quality, stability and safety.

REFERENCES

- Abidi, S. (2003). Tocol-derived minor constituents in selected plant seed oils. *Journal of the American Oil Chemists' Society*, 80, 327-333.
- Abou-Gharbia, H. A., Shahidi, F., Shehata, A. A. Y., & Youssef, M. M. (1996). Oxidative stability of extracted sesame oil from raw and processed seeds. *Journal of Food Lipids*, 3, 59-72.
- Abramovic, H., & Abram, V. (2005). Physico-chemical properties, composition and oxidative stability of *Camelina sativa* oil. *Food Technology and Biotechnology*, 43, 63-70.
- Abramovic, H., Butinar, B., & Nikolic, V. (2007). Changes occurring in phenolic content, tocopherol composition and oxidative stability of *Camelina sativa* oil during storage. *Food Chemistry*, 104, 903-909.
- Abuzaytoun, R., & Shahidi, F. (2006). Oxidative stability of algal oils as affected by their minor components. *Journal of Agricultural and Food Chemistry*, 54, 8253-8260.
- Aghaabasi, K., & Baghizadeh, A. (2012). Investigation of genetic diversity in flaxseed (*Descurainia sophia*) germplasm from Kerman province using inter-simple sequence repeat (ISSR) and random amplified polymorphic DNA (RAPD) molecular markers. *African Journal of Biotechnology*, 11, 10056-10062.
- Ahmadi, L., & Shahmir, F. (2016). Physical Characteristics of Peanut Butter Influenced by Fully Hydrogenated Flaxseed Seed Oil (*Descurainia sophia* L.) as a Stabilizer. *Journal of the American Oil Chemists' Society*, 93, 743-746.
- Ahmadian, M., Duncan, R. E., Jaworski, K., Sarkadi-Nagy, E., & Sook Sul, H. (2007). Triacylglycerol metabolism in adipose tissue. *Future Lipidology*, 2, 229-237.
- Akoh, C. C. (Ed.). (2017). *Food lipids: chemistry, nutrition, and biotechnology*. CRC press. Boca Raton, FL, USA.
- Alamed, J., Chaiyasit, W., McClements, D. J., & Decker, E. A. (2009). Relationship between free radical scavenging and antioxidant activity in foods. *Journal of Agricultural and Food Chemistry*, 57, 2969-2976.
- Ali, M., Gafur, M., Rahman, M., & Ahmed, G. (1985). Variations in fat content and lipid class composition in ten different mango varieties. *Journal of the American Oil Chemists Society*, 62, 520-523.
- AOCS. (1990). AOCS. Official methods and recommended practices of the American oil chemists' society. (4th ed.). AOCS Press, Champaign, IL.
- Ara, K. M., Jowkarderis, M., & Raofie, F. (2015). Optimization of supercritical fluid extraction of essential oils and fatty acids from flaxseed (*Descurainia Sophia* L.) seed using response surface methodology and central composite design. *Journal of Food Science and Technology*, 52, 4450-4458.

- Aruoma, O. I. (1998). Free radicals, oxidative stress, and antioxidants in human health and disease. *Journal of the American Oil Chemists' Society*, 75, 199-212.
- Athukorala, Y., Lee, K. W., Song, C., Ahn, C. B., Shin, T. S., Cha, Y. J., Shahidi, F., & Jeon, J. (2003). Potential antioxidant activity of marine red alga *Grateloupia filicina* extracts. *Journal of Food Lipids*, 10, 251-265.
- Ayerza, R. (1995). Oil content and fatty acid composition of chia (*Salvia hispanica* L.) from five northwestern locations in Argentina. *Journal of the American Oil Chemists' Society*, 72, 1079-1081.
- Ayerza, R., & Coates, W. (2000). Dietary levels of chia: influence on yolk cholesterol, lipid content and fatty acid composition for two strains of hens. *Poultry Science*, 79, 724-739.
- Ayerza, R., & Coates, W. (2001). Omega-3 enriched eggs: the influence of dietary α -linolenic fatty acid source on egg production and composition. *Canadian Journal of Animal Science*, 81, 355-362.
- Ayerza, R. H., & Coates, W. (2004). Composition of chia (*Salvia hispanica*) grown in six tropical and subtropical ecosystems of South America. *Tropical Science*, 44, 131-135.
- Ayerza, R., & Coates, W. (2011). Protein content, oil content and fatty acid profiles as potential criteria to determine the origin of commercially grown chia (*Salvia hispanica* L.). *Industrial Crops and Products*, 34, 1366-1371.
- Bakowska-Barczak, A. M., Schieber, A., & Kolodziejczyk, P. (2009). Characterization of Canadian black currant (*ribes nigrum* L.) seed oils and residues. *Journal of Agricultural and Food Chemistry*, 57, 11528-11536.
- Baldioli, M., Servili, M., Perretti, G., & Montedoro, G. F. (1996). Antioxidant activity of tocopherols and phenolic compounds of virgin olive oil. *Journal of the American Oil Chemists' Society*, 73, 1589-1593.
- Beckman, K. B., & Ames, B. N. (1998). The free radical theory of aging matures. *Physiological Reviews*, 78, 547-581.
- Bekker, N. P., Ul'chenko, N. T., & Glushenkova, A. I. (2005). Lipids from *Descurainia sophia* seeds. *Chemistry of Natural Compounds*, 41, 346-347.
- Blackshaw, R., Johnson, E., Gan, Y., May, W., McAndrew, D., Barthet, V., McDonald, T., & Wispinski, D. (2011). Alternative oilseed crops for biodiesel feedstock on the Canadian prairies. *Canadian Journal of Plant Science*, 91, 889-896.
- Blekas, G., Tsimidou, M., & Boskou, D. (1995). Contribution of α -tocopherol to olive oil stability. *Food Chemistry*, 52, 289-294.
- Botelho, P., Galasso, M., & Dias, V. (2014). Oxidative stability of functional phytosterol-enriched dark chocolate. *LWT - Food Science and Technology*, 444-451.

- Bruun-Jensen, L., Skovgaard, I. M., & Skibsted, L. H. (1996). The antioxidative activity of RRR- α -tocopherol vs RRR- δ -tocopherol in combination with ascorbyl palmitate in cooked, minced turkey. *Food Chemistry*, 56, 347-354.
- Budge, S. M., & Parrish, C. C. (2003). FA determination in cold water marine samples. *Lipids*, 38, 781-791.
- Budin, J. T., Breene, W. M., & Putnam, D. H. (1995). Some compositional properties of camelina (*Camelina sativa* L. Crantz) seeds and oils. *Journal of the American Oil Chemists' Society*, 72, 309-315.
- Byrd, S. (2001). Using antioxidants to increase shelf life of food products. *Cereal Foods World*, 46, 48-53.
- Cahill, J. P. (2003). Ethnobotany of chia, *Salvia hispanica* L. (Lamiaceae). *Economic Botany*, 57, 604-618.
- Carlsson, D., Suprunchuk, T., & Wiles, D. (1976). Photooxidation of unsaturated oils: effects of singlet oxygen quenchers. *Journal of the American Oil Chemists Society*, 53, 656-660.
- Capitani, M. I., Spotorno, V., Nolasco, S. M., & Tomás, M. C. (2012). Physicochemical and functional characterization of by-products from chia (*Salvia hispanica* L.) seeds of Argentina. *LWT-Food Science and Technology*, 45, 94-102.
- Cevc, G. (Ed.). (1993). *Phospholipids handbook*. CRC Press. Boca Raton, FL, USA.
- Chan, H. W. S., & Levett, G. (1977). Autoxidation of methyl linolenate: Analysis of methyl hydroxylinolenate isomers by high performance liquid chromatography. *Lipids*, 12, 837-840.
- Chaw, C. (2000). *Fatty Acids in Foods and Their Health Implications, Second Edition*. Marcel Dekker. New York, NY, USA.
- Chicco, A. G., D'Alessandro, M. E., Hein, G. J., Oliva, M. E., & Lombardo, Y. B. (2009). Dietary chia seed (*Salvia hispanica* L.) rich in α -linolenic acid improves adiposity and normalises hypertriacylglycerolaemia and insulin resistance in dyslipaemic rats. *British Journal of Nutrition*, 101, 41-50.
- Christie, W. W. (1973). *Lipid analysis* (Vol. 167). Pergamon press. Oxford, UK.
- Coates, W. (1996). Production potential of chia in northwestern Argentina. *Industrial Crops and Products*, 5, 229-233.
- Commission of the European Communities (2009). Commission regulation (EC) 827/2009. *Official Journal of the European Union*, 52, 12-13.
- Coppen, P. P. (1994). The Use of Antioxidants," In: J. C. Allen and R. J. Hamilton, Rancidity in Foods, 3rd Edition, Blackie Academic and Professional, New York, 84.
- Daryaei, M. (2007). Treatment miracles of plants in Iranian medicine. *Safirardehal Publication. First Printing*, 218. Publication, Tehran

- Das, N., Berhow, M. A., Angelino, D., & Jeffery, E. H. (2014). Camelina sativa defatted seed meal contains both alkyl sulfinyl glucosinolates and quercetin that synergize bioactivity. *Journal of Agricultural Food Chemistry*, 62, 8385-8391.
- da Silva Marineli, R., Moraes, E. A., Lenquiste, S. A., Godoy, A. T., Eberlin, M. N., & Maróstica Jr, M. R. (2014). Chemical characterization and antioxidant potential of Chilean chia seeds and oil (*Salvia hispanica* L.). *LWT-Food Science and Technology*, 59, 1304-1310.
- de Camargo, A. C., Regitano-d'Arce, M. A. B., Biasoto, A. C. T., & Shahidi, F. (2014). Low molecular weight phenolics of grape juice and winemaking byproducts: antioxidant activities and inhibition of oxidation of human low-density lipoprotein cholesterol and DNA strand breakage. *Journal of Agricultural and Food Chemistry*, 62, 12159-12171.
- de Camargo, A. C., Regitano-d'Arce, M. A. B., Gallo, C. R., & Shahidi, F. (2015). Gamma-irradiation induced changes in microbiological status, phenolic profile and antioxidant activity of peanut skin. *Journal of Functional Foods*, 12, 129-143.
- Decker, E. A., Elias, R. J., & McClements, D. J. (Eds.). (2010). *Oxidation in foods and beverages and antioxidant applications: management in different industry sectors*. Elsevier. Oxford, UK.
- deMan, J. M. (1999). *Principles of food chemistry*. (3rd ed., pp. 33-110). Gaithersburg, MD: Aspen Publishers, Inc.
- deMan, J. M., & deMan, J. M. (1999). Color. *Principles of Food Chemistry*, 229-262.
- Dobarganes, M. C., & Velasco, J. (2002). Analysis of lipid hydroperoxides. *European Journal of Lipid Science and Technology*, 104, 420-428.
- Dolya, V. S., Shkurupii, E. N., Podzolkova, T. V., & Kaminskii, N. A. (1973). The seed oils of some species of the family Cruciferae. *Chemistry of Natural Compounds*, 9, 12-14.
- Du Plessis, L. M., & Pretorius, H. E. (1983). Phospholipid composition of some plant oils at different stages of refining, measured by the latroscan-Chromarod method. *Journal of the American Oil Chemists' Society*, 60, 1261-1265.
- Ebong, P. E., Owu, D. U., & Isong, E. U. (1999). Influence of palm oil (*Elaeis guineensis*) on health. *Plant Foods for Human Nutrition*, 53, 209-222.
- Eidhin, D. N., Burke, J., & O'Beirne, D. (2003). Oxidative Stability of ω 3-rich Camelina Oil and Camelina Oil-based Spread Compared with Plant and Fish Oils and Sunflower Spread. *Journal of Food Science*, 68, 345-353.
- Eidhin, D., Burke, J., Lynch, B., & O'Beirne, D. (2003). Effects of dietary supplementation with camelina oil on porcine blood lipids. *Journal of Food Science*, 68, 671-679.
- Eldin, A. (2006). Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. *European Journal of Lipid Science and Technology*, 1051-1061.

- Embuscado, M., & Shahidi, F. (2015). Handbook of antioxidants for food preservation. *A volume in Woodhead Publishing Series in Food Science. UK: Technology and Nutrition*, 251-83.
- Evans, C. D., List, G. R., Moser, H. A., & Cowan, J. C. (1973). Long term storage of soybean and cottonseed salad oils. *Journal of the American Oil Chemists Society*, 50, 218-222.
- Evans, M. V., Turton, H. E., Grant, C. M., & Dawes, I. W. (1998). Toxicity of linoleic acid hydroperoxide to *Saccharomyces cerevisiae*: involvement of a respiration-related process for maximal sensitivity and adaptive response. *Journal of Bacteriology*, 180, 483-490.
- Fakourelis, N., Lee, E., & Min, D. (1987). Effects of Chlorophyll and β -Carotene on the Oxidation Stability of Olive Oil. *Journal of Food Science*, 234-235.
- Floyd, R. A., & Hensley, K. (2002). Oxidative stress in brain aging: implications for therapeutics of neurodegenerative diseases. *Neurobiology of Aging*, 23, 795-807.
- Frankel, E. N. (1980). Lipid oxidation. *Progress in Lipid Research*, 19, 1-22.
- Frankel, E. N. (1984). Lipid oxidation: mechanisms, products and biological significance. *Journal of the American Oil Chemists' Society*, 61, 1908-1917.
- Frankel, E. (1993). Formation of headspace volatiles by thermal decomposition of oxidized fish oils vs, oxidized vegetable oils. *Journal of the American Oil Chemists' Society*, 70, 767-772.
- French, A. N., Hunsaker, D., Thorp, K., & Clarke, T. (2009). Evapotranspiration over a camelina crop at Maricopa, Arizona. *Industrial Crops and Products*, 29, 289-300.
- Frankel, E. N., Neff, W. E., Rohwedder, W. K., Khambay, B. P. S., Garwood, R. F., & Weedon, B. C. L. (1977). Analysis of autoxidized fats by gas chromatography-mass spectrometry: III. Methyl linolenate. *Lipids*, 12, 1055-1061.
- Frohlich, A., & Rice, B. (2005). Evaluation of Camelina sativa oil as a feedstock for biodiesel production. *Industrial Crops and Products*, 21, 25-31.
- Goffman, F. D., Thies, W., & Velasco, L. (1999). Chemotaxonomic value of tocopherols in Brassicaceae. *Phytochemistry*, 50, 793-798.
- Gordon, M. H. (1990). The mechanism of antioxidant action in vitro. In *Food Antioxidants* (pp. 1-18). Springer, Dordrecht.
- Grilo, E. C., Costa, P. N., Gurgel, C. S. S., Beserra, A. F. D. L., Almeida, F. N. D. S., & Dimenstein, R. (2014). Alpha-tocopherol and gamma-tocopherol concentration in vegetable oils. *Food Science and Technology (Campinas)*, 34, 379-385.
- Guan, R., Jiang, S., Xin, R., & Zhang, H. S. (2007). Studies on rapeseed germplasm enhancement by use of cruciferous weed *Descurainia sophia*. *Genetics and Breeding: Genetics and Germplasm*, 1, 261-265.

- HadiNezhad, M., Rowland, O., & Hosseini, F. (2015). The fatty acid profile and phenolic composition of *Descurainia sophia* seeds extracted by supercritical CO₂. *Journal of the American Oil Chemical Society*, 92, 1379-1390.
- Hall, C., & Cuppett, S. (1993). The effects of bleached and unbleached rosemary oleoresins on light-sensitized oxidation of soybean oil. *Journal of the American Oil Chemists' Society*, 70, 477-482.
- Hammilton, R.J. (1994). The chemistry of rancidity in foods. In *Rancidity in Foods*, (J.C. Allen and R.J. Hamilton, eds.) pp. 1-21, Blackie Academic & Professional, Glasgow.
- Hildebrand, D. H., Terao, J., & Kito, M. (1984). Phospholipids plus tocopherols increase soybean oil stability. *Journal of the American Oil Chemists' Society*, 61, 552-555.
- Hrastar, R., Cheong, L., Xu, X., Miller, R. L., & Kosir, I. j. (2011). *Camelina sativa* Oil Deodorization: Balance Between Free Fatty Acids and Color Reduction and Isomerized Byproducts Formation. *Journal of the American Oil Chemists' Society*, 88, 581-588.
- Ichinose, T., Nobuyuki, S., Takano, H., Abe, M., Sadakane, K., Yanagisawa, R., & Shibamoto, T. (2004). Liver carcinogenesis and formation of 8-hydroxy-deoxyguanosine in C3H/HeN mice by oxidized dietary oils containing carcinogenic dicarbonyl compounds. *Food and Chemical Toxicology*, 42, 1795-1803.
- Imbs, A. B., & Pham, L. Q. (1995). Lipid composition of ten edible seed species from North Vietnam. *Journal of the American Oil Chemists' Society*, 72, 957-961.
- İnanç, A. L. (2011). Chlorophyll: Structural Properties, Health Benefits and Its Occurrence in Virgin Olive Oils. *Academic Food Journal*, 9, 26-32.
- Innis, S. M. (2011). Dietary triacylglycerol structure and its role in infant nutrition. *Advances in Nutrition*, 2, 275-283.
- Ixtaina, V. Y., Nolasco, S. M., & Tomas, M. C. (2008). Physical properties of chia (*Salvia hispanica* L.) seeds. *Industrial Crops and Products*, 28, 286-293.
- Ixtaina, V. Y., Nolasco, S. M., & Tomas, M. C. (2012). Oxidative stability of chia (*Salvia hispanica* L.) seed oil: effect of antioxidants and storage conditions. *Journal of the American Oil Chemists' Society*, 89, 1077-1090.
- Ixtaina, V. Y., Martinez, M. L., Spotorno, V., Mateo, C. M., Maestri, D. M., Diehl, B. W. K., Nolasco, S., & Tomás, M. (2011). Characterization of chia seed oils obtained by pressing and solvent extraction. *Journal of Food Composition and Analysis*, 24, 166-174.
- Jardine, D., Antolovich, M., Prenzler, P. D., & Robards, K. (2002). Liquid chromatography-mass spectrometry (LC-MS) investigation of the thiobarbituric acid reactive substances (TBARS) reaction. *Journal of Agricultural and Food Chemistry*, 50, 1720-1724.
- Jung, M. Y., & Min, D. B. (1990). Effects of α -, γ -, and δ -Tocopherols on Oxidative Stability of Soybean Oil. *Journal of Food Science*, 55, 1464-1465.

- Kamal-Eldin, A. (2006). Effect of fatty acids and tocopherols on the oxidative stability of vegetable oils. *European Journal of Lipid Science and Technology*, 108(12), 1051-1061.
- Khalil, A. H., & Mansour, E. H. (1998). Control of lipid oxidation in cooked and uncooked refrigerated carp fillets by antioxidant and packaging combinations. *Journal of Agricultural and Food Chemistry*, 46, 1158-1162.
- Khan, M. A., & Shahidi, F. (2000). Oxidative stability of stripped and nonstripped borage and evening primrose oils and their emulsions in water. *Journal of the American Oil Chemists' Society*, 77, 963-969.
- Khan, M., Xiao, Y., Yu, B., Wang, N., Rasul, A., Yi, F., & Ma, T. (2012). Artabotryside A, a constituent from *Descurainia sophia* (L.) induces cell death in U87 glioma cells through apoptosis and cell cycle arrest at G2/M phase. *Journal of Medicinal Plants Research*, 6, 3754-3765.
- Kim, T. S., Decker, E., & Lee, J. (2012). Effects of chlorophyll photosensitisation on the oxidative stability in oil-in-water emulsions. *Food Chemistry*, 1449-1455.
- Kim, S. K., Kim, Y. T., Byun, H. G., Nam, K. S., Joo, D. S., & Shahidi, F. (2001). Isolation and characterization of antioxidative peptides from gelatin hydrolysate of Alaska pollack skin. *Journal of Agricultural and Food Chemistry*, 49, 1984-1989.
- King, D. L., Hahm, T. S., & Min, D. B. (1993). Chemistry of antioxidants in relation to shelf life of foods. Developments in food science. in *Shelf Life Studies of Foods and Beverages, Chemical, Biological, Physical and Nutritional Aspects* (pp. 629- 705), Elsevier Science, New York, NY, USA.
- King, M. F., Boyd, L. C., & Sheldon, B. W. (1992). Antioxidant properties of individual phospholipids in a salmon oil model system. *Journal of the American Oil Chemists Society*, 69, 545-551.
- Ko, S., Kim, C., Kim, C., Kim, H., Chung, S., Lee, S., & Kim, I. (2003). Changes of vitamin E content in rice bran with different heat treatment. *European Journal of Lipid Science and Technology*, 105, 225-228.
- Labuza, T. P., & Dugan Jr, L. R. (1971). Kinetics of lipid oxidation in foods. *Critical Reviews in Food Science & Nutrition*, 2, 355-405.
- Laguerre, M., Lecomte, J., & Villeneuve, P. (2007). Evaluation of the ability of antioxidants to counteract lipid oxidation: Existing methods, new trends and challenges. *Progress in Lipid Research*, 46, 244-282.
- Lakshminarayana, G., Rao, T. C., & Ramalingaswamy, P. (1983). Varietal variations in content, characteristics and composition of mango seeds and fat. *Journal of the American Oil Chemists' Society*, 60, 88-89.

- Lambelet, P., Saucy, F., & Löliger, J. (1994). Radical exchange reactions between vitamin E, vitamin C and phospholipids in autoxidizing polyunsaturated lipids. *Free Radical Research*, 20, 1-10.
- Lampi, A., Hopia, A., Ekholm, P., & Piironen, V. (1992). Method for the preparation of triacylglycerol fractions from rapeseed and other oils for autoxidation studies. *Lebensmittel-Wissenschaft Technologie*, 25, 386-388.
- Leonard, E. C. (1998): Camelina oil: α -linolenic source. *INFORM*, 9, 830-838.
- Li, J., Liu, X., Dong, F., Xu, J., Zheng, Y., & Shan, W. (2010). Determination of the volatile composition in essential oil of *Descurainia sophia* (L.) Webb ex Prantl (Flixweed) by gas chromatography/mass spectrometry (GC/MS). *Molecules*, 15, 233-240.
- Li, Q., Wang, J., & Shahidi, F. (2016). Chemical characteristics of cold-pressed blackberry, black raspberry, and blueberry seed oils and the role of the minor components in their oxidative stability. *Journal of Agricultural and Food Chemistry*, 64, 5410-5416.
- Li, W., Liu, X., Khan, M. A., Kamiya, Y., & Yamaguchi, S. (2005). Hormonal and environmental regulation of seed germination in flaxweed (*Descurainia sophia*). *Plant Growth Regulation*, 45, 199-207.
- Li, X., & Mupondwa, E. (2014). Life cycle assessment of camelina oil derived biodiesel and jet fuel in the Canadian Prairies. *Science of the Total Environment*. 481, 17-26.
- Linow, F., & Mieth, G. (1976). The fat-stabilizing properties of phosphatides. III: the synergistic action of selected phosphatides. *Die Nahrung*, 20, 19-24.
- Lísa, M., & Holčapek, M. (2008). Triacylglycerols profiling in plant oils important in food industry, dietetics and cosmetics using high-performance liquid chromatography–atmospheric pressure chemical ionization mass spectrometry. *Journal of Chromatography A*, 1198, 115-130.
- Lu, Y., & Foo, L. Y. (2002). Polyphenolics of *Salvia*—a review. *Phytochemistry*, 59, 117-140.
- Mabarouk, A. F., & Dugan, L. R. (1961). Kinetic investigation into glucose, fructose and sucrose activated oxidation of methyl linoleate emulsion. *Journal of the American Oil Chemists' Society*, 53, 692-695.
- Marsalkienė, N., Sliesaravičius, A., Karpavičienė, B., & Dastikaitė, A. (2009). Oil content and fatty acids composition of seeds of some Lithuanian wild crucifer species. *Agronomy Research*, 7, 654-661.
- Metusalach, Brown, J. A., & Shahidi, F. (1999). Effects of stocking density on composition and performance of reared Arctic charr (*Salvelinus alpinus*). *Journal of Aquatic Food Product Technology*, 8, 39-57.
- Min, D. B., & Boff, J. M. (2002). Lipid oxidation of edible oil. *Food Science and Technology-New York-Marcel Dekker-*, 335-364.

- Miraliakbari, H., & Shahidi, F. (2008). Oxidative stability of tree nut oils. *Journal of Agricultural and Food Chemistry*, 56, 4751-4759.
- Mistry, B. S., & Min, D. B. (1988). Prooxidant effects of monoglycerides and diglycerides in soybean oil. *Journal of Food Science*, 53, 1896-1897.
- Mohd Ali, N., Yeap, S. K., Ho, W. Y., Beh, B. K., Tan, S. W., & Tan, S. G. (2012). The promising future of chia, *Salvia hispanica* L. *Journal of Biomedicine and Biotechnology*, 10, 1-9.
- Moser, R. B. (2010). Camelina (*Camelina sativa* L.) oil as a biofuels feedstock: Golden opportunity or false hope?. *Lipid Technology*, 22, 270-273.
- McVay, K. A., & Lamb, P. F. (2008). Camelina production in Montana. Montana State. Moser, B. R. (2010). Camelina (*Camelina sativa* L.) oil as a biofuels feedstock: Golden opportunity or false hope. *Lipid Technology*, 22, 270-273.
- Moser, B. R., & Vaughn, S. F. (2010). Evaluation of alkyl esters from Camelina sativa oil as biodiesel and as blend components in ultra-low-sulfur diesel fuel. *Bioresource Technology*, 101, 646-653.
- Moreau, R. A., Whitaker, B. D., & Hicks, K. B. (2002). Phytosterols, phytosteranols, and their conjugates in foods: structural diversity, quantitative analysis, and health-promoting uses. *Progress in Lipid Research*, 41, 457-500.
- Muñoz, L. A., Cobos, A., Diaz, O., & Aguilera, J. M. (2013). Chia seed (*Salvia hispanica*): an ancient grain and a new functional food. *Food Reviews International*, 29, 394-408.
- Naczki, M., & Shahidi, F. (2004). Extraction and analysis of phenolics in food. *Journal of Chromatography A*, 1054, 95-111.
- Neff, W. E., List, G. R., & Byrdwell, W. C. (1999). Effect of triacylglycerol composition on functionality of margarine basestocks. *LWT-Food Science and Technology*, 32, 416-424.
- Neff, W. E., Mounts, T. L., & Rinsch, W. M. (1997). Oxidative stability as affected by triacylglycerol composition and structure of purified canola oil triacylglycerols from genetically modified normal and high stearic and lauric acid canola varieties. *LWT-Food Science and Technology*, 30, 793-799.
- Neff, W., Mounts, T., Rinsch, W., & Konishi, H. (1993). Photooxidation of soybean oils as affected by triacylglycerol composition and structure. *Journal of the American Oil Chemists' Society*, 70, 163-168.
- Neff, W., Mounts, T., Rinsch, W., Konishi, H., & El-Agaimy, M. (1994). Oxidative stability of purified canola oil triacylglycerols with altered fatty acid compositions as affected by triacylglycerol composition and structure. *Journal of the American Oil Chemists' Society*, 71, 1101-1109.

- Nijveldt, R. J., van Nood, E., van Hoorn, E. C., Boelens, P. G., van Norren, K., & van Leeuwen, P. A. (2001). Flavonoids: a review of probable mechanisms of action and potential applications. *The American Journal of Clinical Nutrition*, 74, 418-425.
- Nwosu, C. V., Boyd, L. C., & Sheldon, B. (1997). Effect of fatty acid composition of phospholipids on their antioxidant properties and activity index. *Journal of the American Oil Chemists' Society*, 74, 293-297.
- Ohshima, T., Fujita, Y., & Koizumi, C. (1993). Oxidative stability of sardine and mackerel lipids with reference to synergism between phospholipids and α -tocopherol. *Journal of the American Oil Chemists' Society*, 70, 269-276.
- Omura, K. (1995). Antioxidant synergism between butylated hydroxyanisole and butylated hydroxytoluene. *Journal of the American Oil Chemists' Society*, 72, 1565-1570.
- Palma, F., Donde, M., & Lloyd, W. R. (1947). Fixed oils of Mexico. *Journal of the American Oil Chemists' Society*, 24, 27-28.
- Pawongrat, R., Xu, X., & Aran, H. (2007). Synthesis of monoacylglycerol rich in polyunsaturated fatty acids from tuna oil with immobilized lipase AK. *Food Chemistry*, 104, 251-258.
- Peng, L., Yi, Y., Fu-Li, G., & Ze-qü, L. (1997). A preliminary study on the introduction of *Descurainia sophia*, an oil plant species for industrial uses. *Acta Botanica Sinica*, 39, 447-479.
- Pimpa, B., Kanjanasopa, D., & Boonlam, S. (2009). Effect of addition of antioxidants on the oxidative stability of refined bleached and deodorized palm olein. *Kasetsart Journal (Nat Sci)*, 370-377.
- Plessers, A. G., McGregor, W. G., Carson, R. B., & Nakoneshny, W. (1962). Species trials with oilseed plants: ii. Camelina. *Canadian Journal of Plant Science*, 42, 452-459.
- Porras-Loaiza, P., Jimenez-Munguia, M. T., Sosa-Morales, M. E., Palou, E., & LopezMalo, A. (2014). Physical properties, chemical characterization and fatty acid composition of Mexican chia (*Salvia hispanica* L.) seeds. *International Journal of Food Science & Technology*, 49, 571-577.
- Porter, N. A., Caldwell, S. E., & Mills, K. A. (1995). Mechanisms of free radical oxidation of unsaturated lipids. *Lipids*, 30, 277-290.
- Princen, H. M., van Duyvenvoorde, W., Buytenhek, R., van der Laarse, A., van Poppel, G., Gevers Leuven, J. A., & van Hinsbergh, V. W. (1995). Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women. *Arteriosclerosis, Thrombosis, and Vascular Biology*, 15, 325-333.
- Putnam, D. H., Budin, J. T., Field, L. A., & Breene, W. M. (1993). Camelina: a promising low-input oilseed (pp. 314-322). *New Crops*. Wiley, New York.

- Qi, J., Wang, X., Shin, J., Lee, Y., & Jang, Y. (2015). Relative oxidative stability of diacylglycerol and triacylglycerol oils. *Journal of Food Science*, 510-514.
- Rahman, M. J., de Camargo, A. C., & Shahidi, F. (2017). Phenolic and polyphenolic profiles of chia seeds and their in vitro biological activities. *Journal of Functional Foods*, 35, 622-634.
- Rahman, M.J., de Camargo, A. C., Shahidi, F. (2018). Phenolic profiles and antioxidant activity of defatted camelina and sophia seeds. *Food Chemistry*, 240, 917-925.
- Rahmani, M., & Csallany, A. S. (1991). Chlorophyll and β -carotene pigments in Moroccan virgin olive oils measured by high-performance liquid chromatography. *Journal of the American Oil Chemists Society*, 68, 672-674.
- Ramadan, M. F., Kroh, L. W., & Morsel, J. T. (2003). Radical scavenging activity of black cumin (*Nigella sativa* L.), coriander (*Coriandrum sativum* L.), and niger (*Guizotia abyssinica* Cass.) crude seed oils and oil fractions. *Journal of Agricultural and Food Chemistry*, 51, 6961-6969.
- Ramarathnam, N., Osawa, T., Ochi, H., & Kawakishi, S. (1995). The contribution of plant food antioxidants to human health. *Trends in Food Science & Technology*, 6, 75-82.
- Reyes-Caudillo, E., Tecante, A., & Valdivia-López, M. A. (2008). Dietary fibre content and antioxidant activity of phenolic compounds present in Mexican chia (*Salvia hispanica* L.) seeds. *Food Chemistry*, 107, 656-663.
- Rincon-Cervera, M. A., Valenzuela, R., Hernandez-Rodas, M. C., Barrera, C., Espinosa, A., Marambio, M., & Valenzuela, A. (2016). Vegetable oils rich in alpha linolenic acid increment hepatic n-3 LCPUFA, modulating the fatty acid metabolism and antioxidant response in rats. *Prostaglandins, Leukotrienes and Essential Fatty Acids (PLEFA)*, 111, 25-35.
- Salas, J. J.; Sánchez, J.; Ramli, U. S.; Manaf, A. M.; Williams, M., & Harwood, J. L. (2000). Biochemistry of lipid metabolism in olive and other oil fruits. *Progress in Lipid Research*, 39, 151-180.
- Segura-Campos, M. R., Ciau-Solís, N., Rosado-Rubio, G., Chel-Guerrero, L., & Betancur-Ancona, D. (2014). Physicochemical characterization of chia (*Salvia hispanica*) seed oil from Yucatán, Mexico. *Agricultural Sciences*, 5, 220-226.
- Senanayake, S., & Shahidi, F. (2002). Chemical and stability characteristics of structured lipids from borage (*borago officinalis* L.) and evening primrose (*oenothera biennis* L.) oils. *Journal of Food Science*, 67, 2038-2045.
- Scheer, J. F. (2001). *The Magic of Chia: Revival of an Ancient Wonder Food*. Frog Books. USA.
- Shahidi, F. (Ed.). (1997). *Natural Antioxidants: Chemistry, Health Effects, and Applications*. The American Oil Chemists Society. Champaign, IL
- Shahidi, F. (Ed.). (1990). *Canola and rapeseed: production, chemistry, nutrition, and processing technology*. Springer Science & Business Media. New York. US.

- Shahidi, F., & Ambigaipalan, P. (2015). Phenolics and polyphenolics in foods, beverages and spices: Antioxidant activity and health effects—A review. *Journal of Functional Foods*, 18, 820-897.
- Shahidi, F., & de Camargo, A. C. (2016). Tocopherols and tocotrienols in common and emerging dietary sources: Occurrence, applications, and health benefits. *International Journal of Molecular Sciences*, 17, 1745.
- Shahidi, F., & Naczki, M. (2004). Phenolic in food and nutraceutical (pp. 1-558). CRS. USA.
- Shahidi, F., Metusalach & Brown, J. A. (1998). Carotenoid pigments in seafoods and aquaculture. *Critical Reviews in Food Science and Nutrition*, 38, 1-67.
- Shahidi, F., & Shukla, V.K.S. (1996). Nontriacylglycerol constituents of fats and oils *Inform*, (pp. 1227-1231).
- Shahidi, F., & Zhong, Y. (2005). Lipid oxidation: measurement methods. *Bailey's Industrial Oil and Fat Products* (vol. 3, pp. 357-384). John Wiley & Sons, Inc. MA. USA
- Shahidi, F., & Zhong, Y. (2010). Lipid oxidation and improving the oxidative stability. *Chemical Society Reviews*, 39, 4067-4079.
- Shahidi, F., & Zhong, Y. (2011). Revisiting the polar paradox theory: a critical overview. *Journal of Agricultural and Food Chemistry*, 59, 3499-3504.
- Shahidi, F., & Zhong, Y. (2015). Measurement of antioxidant activity. *Journal of Functional Foods*, 18, 757-781.
- Shahidi, F., & Wanasundara, J. P. D. (1992). Phenolic antioxidants. *Critical Reviews in Food Science and Nutrition*, 32, 67-103.
- Shahidi, F., & Wanasundara, U. N. (1998). Omega-3 fatty acid concentrates: nutritional aspects and production technologies. *Trends in Food Science & Technology*, 9, 230-240.
- Shahidi, F., & Wanasundara, U. N. (2002). In C. C. Akoh, and D. B. Min (Eds.), *Food lipids: Chemistry, nutrition and biotechnology* (pp. 465-487). Marcel Dekker Inc. New York, NY, USA.
- Shahidi, F., Wanasundara, U. N., & Amarowicz, R. (1994). Natural antioxidants from low-pungency mustard flour. *Food Research International*, 27, 489-493.
- Smith, J., & Hong-Shum, L. (2011). *Food additives data book*. John Wiley & Sons. UK
- Song, J. H., Inoue, Y., & Miyazawa, T. (1997). Oxidative stability of docosahexaenoic acid-containing oils in the form of phospholipids, triacylglycerols, and ethyl esters. *Bioscience, Biotechnology, and Biochemistry*, 61, 2085-2088.
- Soupas, L., Huikko, L., & Lampi, A. (2006). Oxidative stability of phytosterols in some food applications. *European Food Research and Technology*, 222-266.

- Subagio, A., & Morita, N. (2001). Instability of carotenoids is a reason for their promotion on lipid oxidation. *Food Research International*, 34, 183-188.
- Taga, M. S., Miller, E. E., & Pratt, D. E. (1984). Chia seeds as a source of natural lipid antioxidants. *Journal of the American Oil Chemists' Society*, 61, 928-931.
- Tian, F., Decker, E. A., & Goddard, J. M. (2013). Controlling lipid oxidation via a biomimetic iron chelating active packaging material. *Journal of Agricultural and Food Chemistry*, 61, 12397-12404.
- Topham, M., & Prescott, S. (2010). Chapter 145 – Diacylglycerol Kinases. In R. Bradshaw, & E. Dennis, *Handbook of Cell Signaling (Second Edition)* (pp. 1177) Academic Press. New York. USA.
- USDA (2011). National Nutrient Database for Standard Reference, Release 24. Nutrient Data Laboratory Home Page. US. Department of Agriculture, Agricultural Research Service.
- Valenzuela, R., Bascuñán, K., Chamorro, R., Barrera, C., Sandoval, J., Puigredon, C., & Valenzuela, A. (2015). Modification of Docosahexaenoic acid composition of milk from nursing women who received alpha linolenic acid from chia oil during gestation and nursing. *Nutrients*, 7, 6405-6424.
- Valdivia-Lopez, M. A., & Tecante, A. (2015). Chapter two-Chia (*Salvia hispanica*): a review of native mexican seed and its nutritional and functional properties. *Advances in Food and Nutrition Research*, 75, 53-75.
- Vollmann, J., Damboeck, A., Eckl, A., Schrems, H., & Ruckenbauer, P. (1996). Improvement of *Camelina sativa*, an underexploited oilseed. Progress in new crops. *ASHS Press, Alexandria, VA*, 1, 357-362.
- Vollmann, J., Moritz, T., Kargl, C., Baumgartner, S., & Wagentristl, H. (2007). Agronomic evaluation of camelina genotypes selected for seed quality characteristics. *Industrial Crops and Products*, 26, 270-277.
- Wanasundara, U. N., & Shahidi, F. (1997). Positional distribution of fatty acids in triacylglycerols of seal blubber oil. *Journal of Food Lipids*, 4, 51-64.
- Wanasundara, U. N., Shahidi, F., & Jablonski, C. R. (1995). Comparison of standard and NMR methodologies for assessment of oxidative stability of canola and soybean oils. *Food Chemistry*, 52, 249-253.
- Wanasundara, P. K. J. P. D., Shahidi, F., & Shukla, V. K. S. (1997). Endogenous antioxidants from oilseeds and edible oils. *Food Reviews International*, 13, 225-292.
- Warner, K., Frankel, E. N., & Mounts, T. L. (1989). Flavor and oxidative stability of soybean, sunflower and low erucic acid rapeseed oils. *Journal of the American Oil Chemists' Society*, 66, 558-564.

WHO, (1997). Medicinal plants in China (ISBN 92 9061 102 2), Who regional publications, Western Pacific Series No. 2, WHO regional office for the Western Pacific, Manila, Phillippines.

Wong, D. W. (2018). Additives. In Mechanism and Theory in Food Chemistry, Second Edition (pp. 361-380). Springer, Cham. Albany. USA.

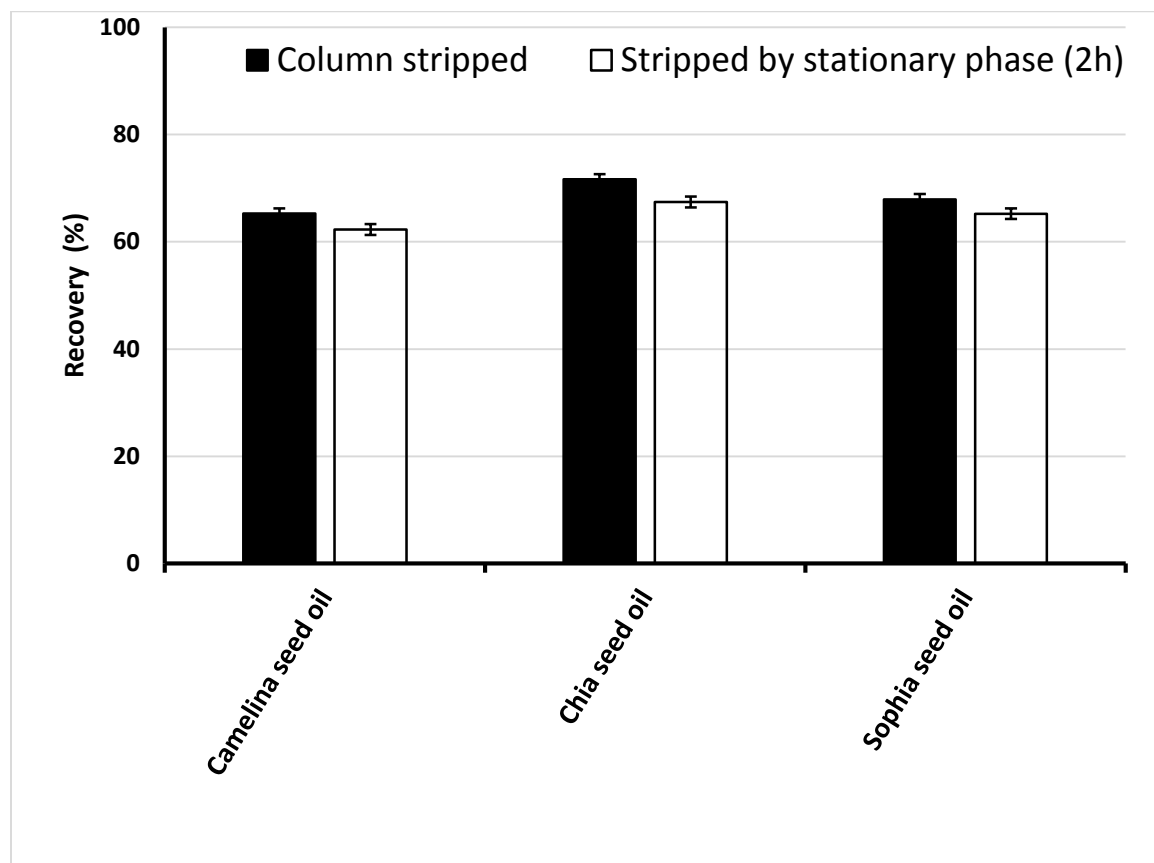
Zhong, N., Cheong, L. Z., & Xu, X. (2014). Strategies to obtain high content of monoacylglycerols. *European Journal of Lipid Science and Technology*, 116, 97-107.

Zubr, J. (1997). Oil-seed crop: Camelina sativa. *Industrial Crops and Products*, 6, 113-119.

Zubr, J. (2009). Camelina oil in human nutrition. *Agro Food Industry HI-Tech*, 20, 22-28.

Zubr, J., & B. Matthaus. (2002). Effects of growth conditions on fatty acids and tocopherols in Camelina sativa oil. *Industrial Crops and Products*, 15, 155-162.

APPENDIX



Graphic presentation of recovery of stripped seed oils as presented in Table

